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TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

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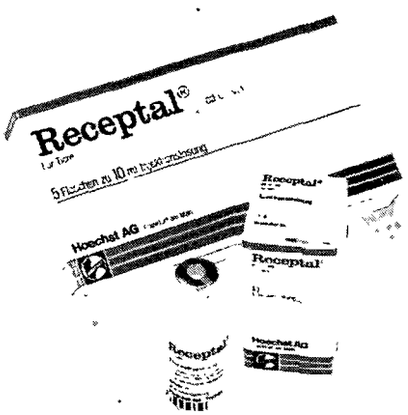

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In the legends to figures Za 2b 3a and 3b of the article DISLOCATION OF THE ELBOW AND ITS SOCIAL CONSEQUENCES FOR AN AFRICAN ELEPHANT by A.I. Hall-Martin and H.P.A. De Boom, this Journal, Vol. 50 No.1 – page 19 (1979 March issue) for "epicondyles" read "condyle".

Index to Advertisers **Advertensie-Opgaaf**

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ADDRESS **TOESPRAAK**

OUR WILDLIFE HERITAGE*

I. MACKENZIE†

Mr President, Ladies and Gentlemen,

It is a privilege for me to have the opportunity of addressing such a distinguished gathering of veterinarians from around the world. It is probably also appropriate for me to be talking to you as a banker for I know it has been said that half a bank's best customers are cats and dogs! There's the true story of the wealthy spinster in London who took her poodle to the bank each Friday afternoon, stood it on the counter and said: "Here you are dear, here is where all *your* money is!"

I would like to share a few thoughts with you this evening on the subject of "Nature Conservation in Perspective". In doing so, may I say at the outset that I feel totally attuned to my audience in the knowledge that I share with you all a profound love of nature in all her facets and, at the same time, extreme concern at the worldwide picture of modern man's potentially fatal impact on the natural world. To quote Prince Philip in "Wildlife Crisis": 'The unpalatable fact about the wildlife crisis is that it is brought about by man. There is no getting away from it; in this disaster, man and man only, is responsible'. For instance, man's more recent scientific and technological advances have led today to a real threat to the ozone layer in the stratosphere which absorbs ultraviolet radiation.

Nuclear weapons, aerosol sprays, supersonic transport, enhancement of food production to feed the world's peoples are all examples of technological advances, but they would not normally be considered in the same context. It now appears, however, that they do have something else in common: they are all potential sources of catalytic agents that penetrate the earth's stratosphere and decompose the ozone that shields living things from the worst of the sun's untraviolet radiation. The full extent of the environmental hazard associated with this phenomenon is still uncertain, despite several years of investigation but it is increasingly clear that a wide range of human activities have the capability of disrupting the delicate photochemical balance on which the earth's ozone buffer, and perhaps life itself, depends.

The United Nations Environment Programme Report says that it would be premature to recommend specific regulations to abate the threat to the ozone layer: the complexities of atmospheric physics and chemistry together with the involved interactions between air, water, soil, plants, animals and man make it exceedingly difficult today to express in quantitive terms the consequences of the human activities mentioned; but, says the report, such uncertainties call for immediate interdisciplinary research in this important area of atmospheric-biospheric science.

On a more poignant, less technical note, was a message to the 1977 World Wilderness Congress here in Johannesburg given by the Red Indian princess, Carol-Ann Brandt. I don't think anyone in the audience that day didn't have a lump in his throat when she read the letter written by Mohawk Chief Seathl to the president of the United States in 1855. He had never heard the words "conservation" or "ecology" but made the most haunting and eloquent plea for them. He wrote: "The great chief in Washington sends word that he wishes to buy our land. The great chief also sends us words of friendship and good will. This is kind of him, since we know he has little need of our friendship in return. But we will consider your offer, for we know if we do not do so, the white man may come with guns and take our land. What Chief Seathl says, the great chief in Washington can count on as truly as our white brothers can count on the return of the season.

My words are like the stars – they do not set. How can you buy or sell the sky – the warmth of the land? The idea is strange to us. We do not own the freshness of the air or the sparkle of the water. How can you buy them from us? We will decide in our time. Every part of this earth is sacred to my people. Every shining pine needle, every shanky shore, every mist in the dark woods, every clearing and humming insect is holy in the memory and experience of my people."

"If I decide to accept, I will make one condition. The white man must treat the beasts of this land as his brothers. I am a savage and I do not understand any other way. I have seen a thousand rotting buffaloes on the prairies, left by the white man who shot them from a passing train. I am a savage and I do not understand how the smoking iron horse can be more important than the buffalo that we kill only to stay alive. What is man without the beasts? If all the beasts were gone, man would die from great loneliness of spirit, for whatever happens to the beast also happens to the man. All things are connected. Whatever befalls the earth befalls the sons of the earth."

Nevertheless, ladies and gentlemen, we must never perpetuate a mood of despondency; rather let us be positive.

My research reveals that the first veterinary college was opened in London in 1791 and that the South African Veterinary Association was founded in 1903 and celebrated its 75th anniversay last year. I know from first-hand experience at my game farm that in your profession it is not enough to like animals; you must also have perserverance, courage, patience, honesty, strength, common sense, a willingness to learn and the ability to turn your hand to anything that needs doing. Quite a daunting list of attributes for the aspiring veterinary surgeon. Not only do you treat animals but you

*Address to SAVA International and National Congress Gala Dinner, 1979.

†Chairman, Standard Bank Investment Corporation Ltd.

also ensure that animal products such as food, skin, wool and hides are free from disease and that infection cannot spread from one area to another, or from animals to humans. You work in laboratories to control disease and in research stations to protect animals and humans. In the context of working with wild life, much more knowledge about the earth and its creatures and the extent of man's environment has yet to be won by your research; spread by education and example and then applied more widely.

Too many times man in Africa has been too quick to rush into nature, imposing an agricultural and industrial approach to produce food when all the time the food in the form of wild animals was there in the first place. He had simply to learn how to use it.

There are approximately 260 million people in Africa south of the Sahara of whom 90 % are rural, directly dependent on ecosystems for their life requirements. Africa has the largest spectrum of wild ungulates in the world which has evolved highly successfully with the full diversity of biomes on the continent – from extreme desert to rain forest – and is adapted to many of the diseases lethal to domestic stock, e.g. nagana transmitted by tsetse flies.

In total there are 91 species of wild ungulates in Africa as compared to only 20 in South America. This unique assemblage of herbivores was, and still is in parts, the protein basis of the peoples of Africa, with the exception of certain groups such as Masai pastoralists. But decimation over vast areas in the name of development and unbridled hunting have reduced this resource, the remnants of which are protected today in national parks. We in South Africa in the karoo area have swopped millions of springbok, believed to be the largest biomass ever, for less than 100 000 sheep.

More than 90 % of Africa suffers from protein and calorie deficiency: the African paradox – dying amidst plenty, what should be an infinite food resource if used on a sustained yield basis.

A perfect example of the industrial approach to land use is the recent (April 1976) irresponsible and indefensible public statement made by FAO experts on the tsetse fly problem. They declare that a massive but long and difficult campaign to eradicate the tsetse fly in Africa could double the total number of cattle on the continent and thus open up "one of the world's greatest potential untapped sources of protein food". This is in the face of bulky species like elephant, buffalo, hippo, eland, giraffe, kudu, etc., which are ready and waiting (having evolved with the system and its parasites) to be utilised on a sustained yield basis. Since their words were spoken the American government has given 12 million dollars to begin the campaign in Botswana.

It is this context that I see the vital role of the veterinarian. The national parks, which have become overcrowded islands threatened by the very species they shelter, be allowed to contribute by restocking suitable areas in Africa. The surrounding people should taste the fruits of translocation and culling operations.

If population is the crux, food is the criterion. Therefore, it is inexcusable to have a veterinary fence contrary to the natural ecosystems and movement of animals causing the death of thousands of animals and resulting in the loss of valuable protein. A more flexible approach and compromise will have to be found.

I am reminded of the words of an African by the name of Nombola, a resident on my farm, who hunted with the great Stevenson-Hamilton, and would be in his nineties today. A man who lived all his life in the wilderness and understands it. Referring to the veterinary fence which runs on the north bank of the Sabi river and cuts off the wild animals from the water and browse at the vital times of the year, he said: "The great spirit has given you a river for the animals to drink from and the succulant browse to feed from and the white man with all his wisdom puts the fence right on the bank of the river and then he wonders why, when the dry time comes, the animals, cut off from water and food, die on the wire. Then when an old man like me comes to take the meat from the dead animals, you arrest me and throw me into jail because you say the meat is not allowed out past the wire and so the vultures and hyenas feed and I go hungry. Why is this nkosaan? Why indeed?"

It follows that if all the wildlife species and their habitats are to survive in Africa and hold their own against the demands of man, they must all become part of a multi-disciplinary form of land use and be subjected to wise use as and when necessary. In other words, we must justify our desire to conserve wildlife and make sure that our terms are compatible with the very demanding needs of developing countries.

Aesthetic and cultural factors are important but it is my conviction that the economic and ecological considerations alone will determine the survival of many of the wildlife areas. Food and economics have high priorities so that wildlife populations can produce worthwhile quantities of protein and earn foreign exchange on a sustained yield basis. An economic justification for conservation is likely to gain wide support.

I was particularly interested to learn that the SAVA has a very active wildlife group and I am indebted to Dr Lynn Colly, the group's secretary, for some information on their activities. The group was inaugurated in 1971 by some 45 vets and it is very heartening to learn from Dr Colly that the number of veterinarians working full-time in the nature conservation field specifically has doubled over the past three to four years. Some highlights of the group's activities have been a seminar in 1972 on 'Wildlife capture techniques and management of captive animals', arranged in conjunction with the University of Pretoria and the wildlife management association. In 1973 the seminar papers were published in book form as the first publication of its kind and this is now in its second printing. Last year saw the first wildlife group study tour incorporating visits to the Lydenburg fish hatchery, Blydepoort and Bourke's Luck, the Hans Merensky Game Reserve and the Kruger National Park. After your present congress, I understand that the group's second study tour will take place, taking in visits to game farms, private game reserves and the Kruger National Park. There is also to be a symposium on all aspects of cheetah ecology, breeding, management and disease with some 14 local and overseas contributors.

On the subject of cheetahs, many of you no doubt read with interest, as I did, the report on the front page of last night's 'Star' newspaper that Aqua, a tame cheetah from the Motswani Game Lodge, near Phalaborwa, is to be fitted with an artificial hip joint. Apparently, the joint was crushed in an accident when she was four months old. This will be the first operation of its kind on a cheetah and I'm sure you all join me in wishing the veterinarian concerned, Dr W. H. Swart from Nel-

spruit, success in this exciting, pioneering operation.

Those deeply concerned with either human or animal welfare easily fall into one of two groups. Those in the one are systematic, sociological, scientific and much concerned with mass remedies and long range plans. The members of the other group tend to be concerned more with the individual case and the need of the moment.

One can only admire the convictions of Phil Drabble, author of "Design for a Wilderness" and "My Beloved Wilderness", who gave up a successful career in the city for a life closer to nature. Many people would associate themselves with him when he said: "Every morning my car got snarled up on the same traffic jams at the same traffic lights. My guts were filled with the same stench of diesel and no bird song could sing loud enough to drown grumbling exhausts and whining gears. The same face seemed to peer through every windscreen, as apathetic and expressionless as every other face. The only signs of animation in these faces were transcient glares of hate at whoever had the initiative to jump the queue"

I am quite sure that the majority of you here this evening are familiar with the delightful reminiscences of James Herriot, the English veterinarian, about the rich memories of his professional life. A host of friends, animal and human, march across the pages, all revealing Herriot himself with his dedication of the job and his unequalled capacity for seeing the silly side of things. How vividly he portrays the doctoring of animals, interwoven as it indeed is with success and failure, humour and sadness. I liked the bit of advice given to him and his fellow students by a professor at university when he said: "If you are going into private practice make sure you open up in a strategic position – preferably right next to the pet shop. You'll get a good spin-off business from the shop and at the same time you can become experts in goldfish". Goldfish, he said, stroking his moustache, suffer from a lot of peculiar, obscure diseases. I couldn't fathom out any of them. What I always did was tell the owners to leave 'em with me overnight, 'For observation'. As soon as they'd done I'd get my girl to slip next door to the shop and buy another fish – same size, same markings and costing only five bob. I was known for miles around as the greatest expert on reviving sick goldfish the good Lord had put breath into".

The struggle to equate the development of human progress with the protection of the global environment and the ever more rapid depletion of natural resources, can only be resolved by wise compromise. Economic growth and social progress cannot be pursued indefinitely at the expense of the quality of life on earth. To continue such a policy can only widen the gulf between the haves and have-nots and lead ultimately to conflict. Development must be fair to all and must at every stage include an element for the improvement of the human condition, or it will be useless. If we are to survive the pressures of the next century, the land and oceans must remain fully productive, our fresh water and our air free of harmful pollution. Wilderness and wildlife must be allowed to survive, for man and nature are totally interdependent and the products of the same evolutionary process. The diversity of life on earth is our richest asset. We destroy it at our peril. It is not sufficient that the United Nations and 130 governments are now pledged to protect the environment. The task is one which can only succeed if every individual feels personally involved and plays his part by restraint and by opening his mind to the realities of human survival in the twenty-first century. Only if the decision makers, and this usually means politicians, can be taught the essential principles of conservation are options likely to be kept open. There is nothing mysterious or difficult to comprehend about these principles. Perhaps the greater difficulty lies in the inability of the decision makers to put the problems of the future on the same plane as the always pressing need for quick results to solve the problems of today.

In order to survive, man must learn to live with, instead of against nature: to conserve instead of destroying or polluting the precious natural resources of the biosphere – the clean air, the fresh water, the oceans, the land, and the living organisms of which his very life depends; to recycle, instead of wasting, all materials used in manufacture; to abandon the theory of built-in obsolescence as a means of stimulating the demand for automobiles and household appliances and to build instead goods which will last. If man is to maintain his equilibrium in a crowded urban existence, he must exchange the tensions of a highly competitive society for one in which materialism is no longer admired. The alternative of attempting to maintain the present headlong course on which the developed countries are embarked, can only lead to a power struggle for control of the dwindling resources of the world, in which, in a nuclear age, there can be no winners.

We all live under a perpetual question mark. But how trifling seem the economists' charts and the bankers' balance sheets, how remote the strife of politics, how barren the quarrels between race and religion, when we behold spring unlock nature's treasure chest as indeed is happening for all to see in our beloved South Africa.

I would like to leave you with the eloquent words of Wallace Stegner, professor of English at Stanford University: "Something will have gone out of us as a people if we ever let the remaining wilderness be destroyed, if we permit the last virgin forests to be turned into comic books and plastic cigarette cases; if we drive the few remaining members of the wild species into zoos or to extinction; if we pollute the last clear air and dirty the last clean streams and push our paved roads through the last of the silence. We simply need that wild country available to us, even if we never do more than drive to its edge and look in, for it can be a means of reassuring ourselves of our sanity as creatures, as part of the geography of hope".

ADDRESS **TOESPRAAK**

SAVA BIENNIEL CONGRESS '79 PRESIDENTIAL ADDRESS AT OPENING CEREMONY

R.I. COUBROUGH

Mr Chairman, Honoured Guests, Colleagues, Ladies & Gentlemen,

In many ways the Veterinary profession is a most remarkable profession. We have often with the odds turned decidedly against us made phenomenal progress, and achieved great heights in our contributions to the development of this country's livestock industry. Our profession's role in the control of rampant infectious disease plagues which formally wrought havoc, has left an indelible mark on the epoch of progress. We stand on the shoulders of great scientists like Sir Arnold Theiler, Alexander & Neitz whose achievements in disease control and erradication have turned the Veterinary eyes of the world toward us. We have frequently been called upon to exhibit an ingenuity, an adaptability, a tenacity and singleness of purpose to contend with the problems which have confronted us, and still do confront us from day to day, as we endeavour to fill our place in the sun. The devotion to duty which exists within this profession is a legacy which finds no peer in other professions.

As a member of a team responsible for the welfare of a multimillion rand industry, the potential of which when reflected upon in terms of a Southern constellation of States has hardly been touched, we perform a most significant function with a leading part to contribute. We have grown from a profession employed largely by the State to one now also employed in a manifold of diversified facits of private enterprise. Each facit of veterinary endeavour while having in itself an inherent right to exist forms part of a whole, committed to fulfil a role in creating a harmoniously growing community. But for optimal functioning, and maximal contribution to animal health be it companion animal or production animal oriented, it is now perhaps more than ever imperative that we as a profession must establish a far greater interaction, ensure a more positive communication and foster a flexibility of purpose. We must guard against each facit of the profession becoming so pre-occupied in doing its own thing that it unwittingly protects what it believes (rightly or wrongly) are its boundries, too jealously – we draw the line – thus far and no further, and tend to go on the defensive instead of examining the synergistic benefit of interaction. We must avoid at all costs living so close to our interest spheres that we lose sight of the realities of the situation. The intensive relook at our profession, at education, at our role in rural practice, city practice and wherever we may be found, a relook initiated at all levels will allow us to stand back and reflect on well reasoned soundly motivated and meaningful change. While we must in no way cast aside our proud traditions, we must not hesitate to initiate change if this will be positive in its effect. Veterinary presence in rural areas is a cornerstone of the live-stock industry, and is one of the regions of veterinary activity under intensive investigation. The highly competitive nature of livestock production today requires, nay demands, a shift of emphasis away from the individual animal towards herd health and preventive medicine schemes that will be economically feasible and eliminate the massive loss in cash and kind which occur each day in the livestock industry – losses which can in terms of the application of modern knowledge and technology be prevented, losses which we as a developed country cannot and should not allow to happen. But the solution will not be forthcoming overnight, will require a concerted effort from veterinarian and farmer alike. We will have to meet each other in frank and open discussion. We will have to ask if the time is not ripe to relook at the age-old question of the establishment of veterinary district surgeons, or at co-operative insurance schemes such as exist (and work) in Israel. But we must not only ask questions – we must actively look for answers – we must become solution seeking not problem orientated. We must look first for the reasons "why" and then the many reasons "why not" which only deviate and negate perspective.

And this relook could not have come at a better or more opportune time – for there is a decided air of pessimism concerning the future of the veterinary profession, which pervades and permeates veterinary ranks, not only here but world wide. It may be but a symptom of the flagging world economy but we cannot (we dare not) allow it to infiltrate our lives any further. Throughout the world there is talk of an over production of veterinarians, a fear which fills the hearts of many in this country too. I for one do not share this pessimism, for what confronts us in this country, is not the dread of an over production of veterinarians, but the millstone of a hopeless under-utilisation. An under-utilisation which has hamstrung real progress to the extent that we may have even come to accept it as the norm. As a developed country we must not only maintain the status quo but must set a pace that will provide a lead – many problems remain untouched because of lack of veterinary man-power: disease eradication programmes (TB, brucellosis) are perhaps not allowed to reach their desired (and required) magnitude and may even be curtailed in attaining set goals; erosion diseases like mastitis continue to erode; neonatal mortality persists at an alarmingly high rate in some areas: all of which have a concommitant massive effect on the gross national product derived from the livestock industry. I believe that if we focus our sights on filling the gulf of underutilisation then we will open a myriad of new opportunities, create new horizons which will herald a dawn of hope for the morrow. We must look beyond the conventional traditional fields of activity, improvise if necessary, create a new mode of approach, set a re-

vised pattern which will help hew a pathway along which a new generation can travel. For the future is solely dependent on the action and direction of the present – that responsibility is ours.

By virtue of our broad based training/education we as a profession are fortunate to be able to occupy a fulcrum position from which we can offer the type of service required by either the companion animal or livestock owner. It is going to be the quality of service which we as a profession can offer against all competition, and within economically acceptable bounds both towards ourselves, as well as to those we serve, which determines our future. For unlike human medicine which in today's world would seem to know no economic bounds veterinary medicine remains (fortunately) fettered within the shackles of economic reality set in terms of practical production limits. This places a ceiling on the inherent value of the forms of life with which we and allied professions have to deal. This in itself must exert a self restraining effect, acutely influenced by the norms of economic livestock production.

One of the major contributing factors of underutilisation of veterinary services, apart from of course sheer lack of manpower, has been a lack of awareness of what can be done by the profession for those we serve. Thus any programme bent on alleviation of the problem must include the projection of an image in professional terms which will engender an awareness. People must know what we are about; people must be told and shown what we as part of a team can offer which cannot be obtained elsewhere. We must establish contact with leaders in the various fields of allied activity who form part of this team. Communication must become a cornerstone in the ultimate success (or failure) of our profession. For without contact there can be little or no understanding. And without understanding, misconception becomes the order of the day, a situation tailored to hamstring progress. The responsibility of creating these channels of communication and thereby projecting a positive professional image again rests with all of us.

As a profession we have a most definite identity, which perhaps we have not been able – or always been permitted to live out to the full. It is only natural that in the present position of the profession within governmental hierachy that at times our interests may be overshadowed or even engulfed by the overall problems of other divisions that drink from the same fountain. It would I believe be expedient and in the interests of the livestock industry, in general, and the country as a whole to re-investigate the feasibility of creating a situation of greater autonomy and independence. This in no way suggests that as a profession we can go it alone – for it is a well established fact that we are a member of a team whose common concern is the welfare of our animal population. But many facits of overall disease control and eradication require a special approach which may not always be understood by those at the top despite sound and adequate motivation of the project. Disease eradication schemes, especially those involving zoonoses, are priority situations which necessitate individual attention and specific funding not always permissable or forthcoming under the present conditions. It would thus perhaps be irresponsible not to broach this subject once more with the relevant authorities. While not being insensitive to the economic implications, these would in the long term be offset by the positive contribution forthcoming to animal and human health. This requires a bold well motivated and sincere approach and offers a challenge we must not ignore or overlook because we have been rebuffed before.

Any possible changes envisaged must always take into account the nature of the supportive infrastructure. For the ultimate success and optimal functioning of any profession will depend on a solid, well trained and content infrastructure. While we as a profession have a well established infrastructure to which we have just added veterinary nurses: the time is perhaps ripe to review the situation from a global point of view. We must ensure an adequate, well balanced and specific training programme, and just as we seek a future for ourselves, create the same hopes for the aspirations of those who form the infrastructure of the profession. We must not fear that any upgrading of this infrastructure will erode our work sphere, for a far grater danger lies in the under trained situation. As part of a team this infrastructure forms an integral part of our future and will create opportunity and contribute to a more efficient utilisation of the available resourses serving the animal industry.

We as a profession both here, and elsewhere in the world, stand on the threshold of a new adventure. We must seek a dream, a new vision, mould an ideal. It will not just happen, we will have to make it work. We must ask ourselves, and seriously too, whether we believe that there is a future for the veterinary profession; whether we believe we still have a contribution to make to the welfare of society.

Our presence here today I am sure, is witness to the fact that we do believe there is a future. In academic fare of such wide a taste, there must surely be a spark that will kindle the flame within and engender a new spirit of life and hope into our beloved profession. A stimulus we can take to the far corners of the world creating a new enthusiasm amongst our profession which will radiate outwards to all those that we endeavour to serve, thereby creating an awareness of our worth in companion animal care, and in an economically orientated livestock production programme.

CRETINISM IN ANGORA GOATS

G.F. BATH*, D. WENTZEL†, and E.M. VAN TONDER*

ABSTRACT: Bath G.F.; Wentzel D.; van Tonder E.M. **Cretinism in Angora goats.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 237–239 (En) Regional Veterinary Laboratory, Private Bag X528, 5900 Middelburg, Rep. of South Africa.

An outbreak of goitre and hypothyroidism in newborn Angora kids is described. The does had been grazing on lucerne from the time of mating and received a free-choice lick, which included iodine. Investigations revealed that the condition was iodine-responsive, and was probably caused by a goitrogen like thiocyanate.

INTRODUCTION

Neonatal goitre is a well known phenomenon which has been described in most domestic animals[2,3,5–8] but to our knowledge not in Angora goats. Hypothyroidal goitre is generally accepted as being the result of one of three broad aetiological categories. The first is a frank or marginal deficiency of iodine in the diet. In the latter case some additional factor is needed to present obvious symptoms. The second category encompasses all goitrogens which either interfere with the uptake of iodine from the diet, or with its metabolism in the formation of thyroxin within the body. Many goitrogenic substances have been described, one of the most important of which is thiocyanate which has frequently been incriminated in outbreaks of goitre in grazing animals[1,5–8]. Its action can be overcome by additional iodine since it acts by inhibiting the selective concentration of iodine by the thyroid[8].

By contrast, another important goitrogen (goitrin or thiooxalidone) which is found in kale, blocks thyroid hormonogenesis and its effects cannot be overcome by feeding extra iodine[8]. There are also many other known goitrogens which include thiouracil, carbimazole and perchlorates.

The third aetiological group includes all forms of defects in the genesis of thyroxin, and are therefore nearly always hereditary[2,4,7,8].

Apart from goitre, the most frequently described signs of neonatal hypothyroidism (cretinism) are dwarfing, obesity, electrolyte disturbances, hair loss and skin changes, subsequent infertility, skeletal malformations, mental retardation and sluggishness[5–8].

HISTORY

The Angora goats originated from a farm in the Camdeboo area of the Graaff-Reinet district. The owner had kept Angoras many years previously but had not seen goitre in them before, nor had he ever seen it in sheep or cattle on the farm. He did not know of any neighbours who had experienced the same problem.

The does had been bought two years before from several distant districts. The year previous to the outbreak there were no cases of goitre seen in kids born to does grazing on the same pastures, though the kidding rate in does was rather low. The does at the outbreak were in two groups and had been put onto irrigated, fertilised lucerne pastures from the time of mating and were not removed at any stage. For three months until shortly before kidding they had access to a free choice system of trace element supplementation. This was in the form of a special apparatus which protected hard blocks of various salts against weather conditions. According to the labels these 15 blocks contained respectively manganese, iron, cobalt, copper, zinc, molybdenum, calcium, magnesium, phosphorus, iodine, sodium, chloride, potassium, sulphur and aluminium in various salts.

Of the two groups of does on separate pastures, 3 kids born from a group of 115 does and 5 kids born from 183 does showed very clear goitre, while several more were mildly affected on closer examination. Nearly all goitrous kids were obviously affected from birth. At the time of presentation they were already several weeks old.

CLINICAL EXAMINATION AND AUTOPSY FINDINGS

All affected kids were stunted in stature and had a blocky appearance. The faces were shortened anteroposteriorly and apparently broadened laterally. The eyes appeared to bulge sideways but as the lids drooped exophthalmos was not observed. Prognathia inferior was present in all cases.

The kids were clearly much fatter than unaffected ones and were dull and inactive. Goitre was easily palpable bilaterally in all cases, in some the enlargement of the thyroid glands was gross and could be clearly seen in spite of the presence of long hair. There was no

Fig. 1. A typical case of cretinism in an Angora kid.

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sign of hair loss, hair thinning or skin abnormality (Fig. 1). Pathological changes apart from previously described lesions included gross obesity and slight subcutaneous oedema. The thyroids were firm, brown and mainly enlarged in the lobes while the isthmus was virtually unaffected. One gland weighed 83,4 g compared to the throid of a normal but slightly older goat, which weighed 2,5 g.

Both kidneys and liver were very pale in nearly all cases. Atrophy of lymphoid tissues and gastrointestinal stasis were also constant findings. The latter could possibly be related to separation of kids from their dams at a time when they were still heavily dependant on goats milk, even though bovine milk was given.

SPECIAL INVESTIGATIONS

Histopathology

Formalin-fixed specimens were stained HE. Changes in the thyroids were consistent with those described in cretinous goitre[7] (Fig 2). The paleness seen grossly in livers and kidneys was found to be due to fatty infiltration while in the kidneys hyaline droplets or casts in some tubuli were seen.

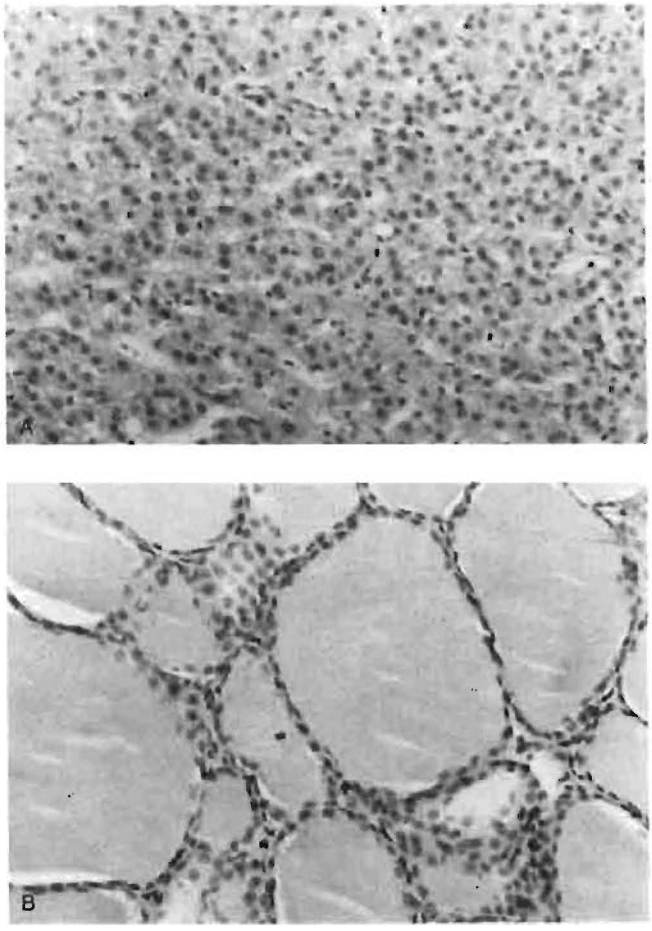

Fig. 2. A: Microscopic appearance of the thyroid showing marked hyperplasia and decreased, irregular acini; HE x 400.
B: Normal thyroid HE x 400.

Chemical pathology

Plasma cholesterol of affected kids was over 5 times higher than randomly bled Angora kids which came from the Grootfontein Agricultural College (Table 1).

Table 1: PLASMA CHOLESTEROL AND THYROXINE LEVELS IN GOITROUS AND NORMAL GOATS

Subjects		Cholesterol mmol/ℓ	Thyroxin μg/dℓ
Goitrous goats	1	7,1	2,7
	2	18.0	2.4
	3	8,0	3,4
	4	10,5	3,9
	Mean	10,9	3,1
Normal goats	1	2,1	6,9
	2	2,3	5,3
	3	1,6	5,4
	4	1,1	6,0
	Mean	1,8	5,9

Thyroxin determination[9] revealed levels in affected kids nearly half those of normal controls (Table 1).

Thyroid uptake of radioiodine and its clearance rate from the blood was measured in 2 goitrous goats from the farm and 2 young Grootfontein Angora goats by injecting 25 μ Ci ^{125}I intravenously. Blood was withdrawn from the opposite jugular to measure radioactivity by scintillation counter at predetermined intervals. In addition, since urine was the chief route of excretion of iodine[8], urine was measured in the same way for radioactivity over the 24 hours following administration. The actual uptake of ^{125}I by the thyroid gland was measured by means of a directional counter. It is clear from the accompanying graph (Fig. 3) that goitrous

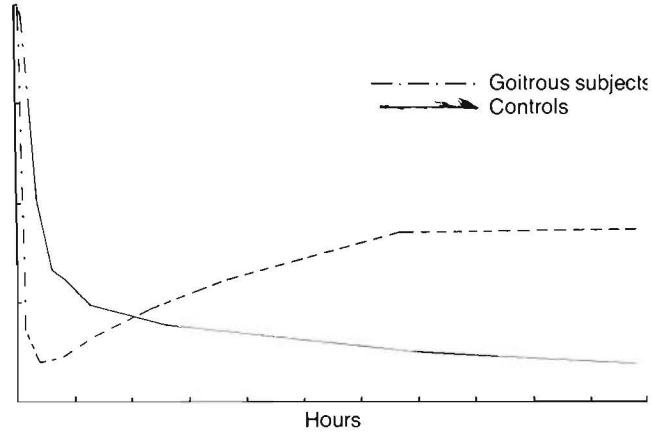

Fig. 3. Changes in plasma radioactivity after intravenous injection of ^{125}I into goitrous and normal goats.

subjects cleared injected radioiodine much more rapidly from the blood within the first two hours of administration than did normal controls. While circulating ^{125} continued to drop in normal subjects, as would be expected, there was a secondary sustained rise in goitrous individuals between 1,5 and 13,5 h after administration of the isotope. This strongly suggests that the radioiodine was rapidly taken up by the thyroid and equally rapidly used in the formation of thyroid hormone which was then released into the blood stream. Additional evidence of rapid isotope uptake by the thyroid was evident in the radioactivity level of the thyroids, which was found to be 53 times higher in the goitrous group than in the control group. Urinary concentration of radioiodine was 81,94 million counts in normal goats as against 4,03 million counts in affected ones. This again indicates tenacious retention of injected iodine by goitrous individuals.

DISCUSSION

The rapid uptake of radioiodine by the thyroid, its rapid clearance from and then reappearance in the blood, and its low level in urine in goitrous goats compared to normal controls, rule out the possibility of an iodine-trapping or iodine-losing metabolic defect. It is quite clear that the condition was iodine-responsive and that only the way in which iodine deficiency was created remains in doubt. While a straightforward iodine deficiency was not specifically excluded by analysis of the grazing, it is unlikely that the deficiency of iodine could have been anything more than marginal. It could certainly not alone account for the sudden onset of severe goitre and hypothyroidism in only one farm in the area and one species on that farm. Mild cases of goitre in young goats without other signs of clinical hypothyroidism have been seen occasionally in the district and indicate the possibility of a marginal deficiency.

It seems therefore that the condition was largely caused by one or other goitrogen which was not specifically identified. Because its effects cannot be overcome by supplemental iodine, goitrin or a similar substance has been considered unlikely. Of the known goitrogens which affect grazing animals, thiocyanate seems by far the most likely goitrogen which could account for the outbreak. The precursors of thiocynate are cyanogenetic glycosides which are present in a wide variety of plants[1 6 8], particularly when the plants are highly fertilised[5 6 8]. The fertilisation level followed by the farmer was far higher than any other in surrounding district, and could explain why only his farm was affected.

The question of the possible effect of the free choice lick also deserves comment. While the range offered included iodine as one of the components, its presence *ad libitum* for 3 months during the late stages of gestation did not prevent the occurrence of severe iodine-responsive goitre in new-born kids. The previous year, in goats grazing on the same land but not receiving a free choice lick, no cases of goitre were recorded. It is therefore clear that the free choice availability of a range of elements including iodine not only failed to prevent the development of an iodine deficiency (whether primary or secondary) but also may have even aggravated the condition. Three minerals included in the range, viz. calcium, cobalt and manganese, have been shown to or suspected of having an interrelationship with iodine metabolism[8] while interrelationships with other elements can by no means be ruled out. These observations lend support to the opinion that animals do not have sufficient nutritional wisdom to properly balance their requirements of various nutritional components, including trace elements.

ACKNOWLEDGEMENTS

We thank the Director of Veterinary Field Services and the Director, Karoo Region for the opportunity and facilities to undertake this investigation. We also thank the technical staff of the Regional laboratory and the Research Institute for their assistance in carrying out the investigation.

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LETTER TO THE EDITOR

BRIEF AAN DIE REDAKSIE

Dear Sir,

All members of our Association who have not yet had the privilege of attending a meeting of the Council will be unaware of the dignity of the proceedings.

My short exposure to this body has given me added pride in our profession and to see the manner in which these 30-odd veterinarians devote four Saturdays a year has caused me to regain faith in mankind's better side. The unpaid time they spend at Council meetings is but a fraction of the energy devoted by our office bearers to the good of our profession. They deserve our gratitude.

Yours faithfully,

Desmond Irwin,
P.O. Box 4107,
Alrode.

'N VEILIGE TEGNIEK VIR DIE INSNYDING VAN DIE HORINGVLIES MET OPPERVLAKKIGE KERATEKTOMIE

S W PETRICK

ABSTRACT: Petrick S.W., **A safe technique for the incision of the cornea with superficial keratectomy.** *Journal of the South African Veterinary Association.* (1979) **50** No. 4 240 (Afr. en.) Dept. Surgery, Fac. Vet. Science, Univ. Pretoria, P.O. Box 12580, 0110 Onderstepoort, Rep. South Africa.

The technique, illustrated by photographs, allows superficial keratectomy to be done simply and without danger of perforating the cornea, inasmuch as a No. 15 surgical scalpel blade is used, clamped in a curved Halstead mosquito artery forceps, so that no more than 0.5 mm of the blade protrudes. In over 50 corneal incisions no complications were experienced. In two cases the operation had to be repeated after adjustment of the blade to allow a slightly deeper incision to be made.

INLEIDING

Oppervlakkige keratektomie behoort 'n baie meer algemene operasie te wees, veral in die Alsatian, met gepigmenteerde keratitis, pannus en korneale distrofie wat geredelik voorkom.

Vir die ongeoefende hand blyk die korneale insnyding die knelpunt te wees, weens die vrees van 'n perforasie.

'n Eenvoudige en veilige tegniek word hier beskryf vir genoemde insnyding.

INSTRUMENTASIE EN TEGNIEK

Die volgende instrumente word benodig:

a Geboë Halstead muskietaarklem van 12,5 cm lank, of 'n soortgelyke klem.

b 'n Nr. 15 chirurgiese lem.

Die lem word vasgeklem in die aarklem sodat die sigbare snykant van die lem nooit meer as 0,5 mm is nie (Fig 1). (Die dikte van 'n hond se horingvlies is net minder as 1 mm). Met die instrument dan vasgehou deur die eerste drie vingers van die hand (Fig. 2) word met 'n enkele gladde beweging van insnyding wat orals ewe diep is, oor die horingvlies voltooi.

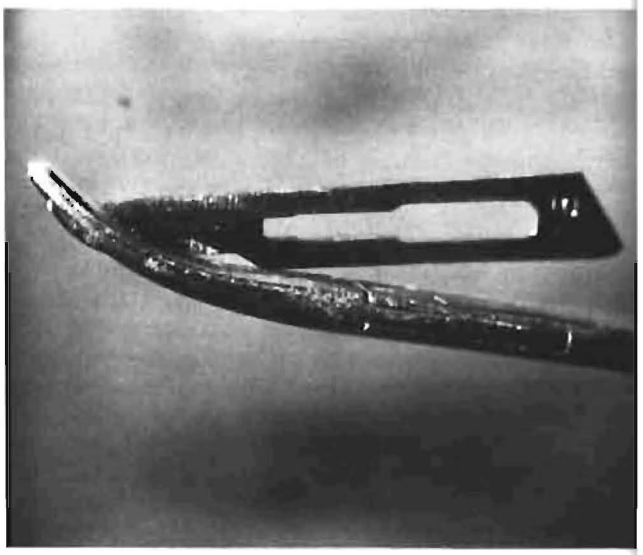

Fig. 1. Die nr. 15 lem vasgeklem in 'n geboë Halstead-muskietaarklem.

RESULTAAT

Met meer as 50 horingvliesinsnydings op die wyse reeds voltooi, was daar geen perforasies nie.

In twee gevalle waar die keratektomie nie al die patologiese weefsel verwyder het nie, is na verstelling van die lem 'n tweede insnyding uitgevoer en die oorblywende weefsel verwyder.

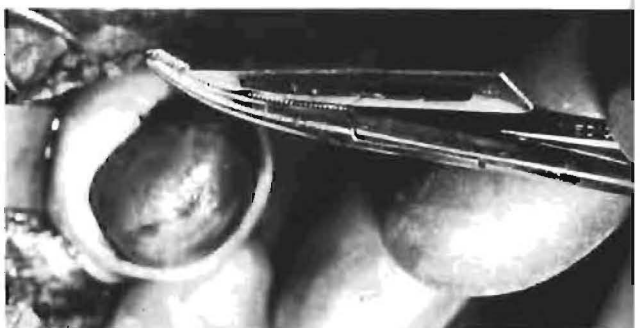

Fig. 2. Die oogbal word geprolabeer: die beskute lem is gereed vi die insnyding.

GEVOLGTREKKING

Dit is 'n eenvoudige en veilige metode om 'n horingvliesinsnyding suksesvol uit te voer.

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THE USE OF DOXYCYCLINE IN THE TREATMENT OF CANINE EHRLICHIOSIS

J. VAN HEERDEN* and A. IMMELMAN†

ABSTRACT: Van Heerden, J.: Immelman, A. **The use of doxycycline in the treatment of canine ehrlichiosis.** *Journal of the South African Veterinary Association.* (1979) **50** No. 4 241-244 (En) Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Rep. of South Africa

The use of doxycycline in the treatment of twenty dogs with canine ehrlichiosis is described. The drug was found to be effective even in cases which did not respond to treatment with oxytetracycline.

INTRODUCTION

Canine ehrlichiosis or tropical canine pancytopaenia is being encountered with increased frequency in the Department of Medicine, Faculty of Veterinary Science, University of Pretoria. The symptoms and clinical pathological findings of the disease have been described[2,4]. Specific chemotherapy[4] consists of the intravenous administration of oxytetracycline at a dose rate of 10 mg/kg once daily for at least ten days. Immelman[3] has shown that a single oral dose of 100 mg/kg once daily or in divided dose twice daily yields a blood level of 1μg/ml of oxytetracycline for 24 hours. This dose rate has been routinely and effectively used in the treatment of canine ehrlichiosis in our clinic. Despite initial good results, relapses have occurred and this has prompted investigations with other drugs. Doxycycline is a synthetic derivative of methacycline and is also known as alpha-6-deoxy-5-oxyetetracycline hydrochloride. The mode of action is similar to the other tetracyclines, namely the inhibition of bacterial protein synthesis. Doxycycline differs from the other tetracyclines in having a high lipid solubility as well as being more completely absorbed from the gastro-intestinal tract than oxytetracycline. After absorption the antibiotic is 30 % protein bound and penetrates well into tissues. Penetration of the blood brain barrier is negligible. Renal clearance of doxycycline is lower than that of oxytetracycline. This could possibly be due to the higher lipid solubility which results in increased reabsorption from the kidney tubules. Due to the high absorption and slow excretion rates, doxycycline has a long serum half life, approximately 19,5 h in comparison to 9,5 h in the case of oxytetracycline. The advantage of this is a smaller dose and longer interval between doses[1,5].

This report deals with the use of doxycycline in canine ehrlichiosis.

MATERIALS AND METHODS

Capillary blood smears were stained with Quik-stain§ and examined for the presence of E. canis.

Twenty dogs in which ehrlichiosis had been diagnosed were treated either intravenously or orally with doxycycline‡ at dose rates varying from 4,6 to 28,5 mg/kg (Table 1). In the case of oral administration the drug was given once daily for ten days and in the case of intravenous administration daily treatment was continued for five days.

In certain cases chemotherapy was combined with supportive therapy which consisted of blood and fluid administration§. Concommitant infection with *Babesia canis* was treated with phenamidine‡.

The cases were classified into three groups namely "acute", "chronic" and "biliary relapse". It should be noted that this grouping is somewhat arbitrary and did not always accurately reflect on the course of the disease.

"Acute" cases were mainly characterized by severe depression and anaemia. Morulae of *Ehrlichia canis* were found in monocytes (Fig. 1) in all cases except one in which granules only, were found in the cytoplasm of monocytes (Fig. 2).

"Chronic" cases included all cases (except one: No 18) that were treated unsuccessfully with oxytetracyclines at the usually effective dose rate of 100 mg/kg given either orally and/or intravenously. These cases were characterized by weight loss and listlessness. In all but 2 cases (No 18 & 20) morulae were demonstrated in monocytes. Only two dogs showed severe epistaxis (see Table 1 – No 17 and 18). The diagnosis in cases No 18 & 20 was based on clinical signs and the presence of leucopeania and thrombocytopenia.

The "biliary relapse" cases had all been diagnosed as babesiosis some two to five weeks prior to admittance to the Department of Medicine. Only one case in this group (No 12) showed severe anaemia, emaciation, and marked epistaxis.

Blood was collected in EDTA vacuum tubes and the red and white cell count as well as haematocrit were determined with the aid of a Coulter-Counter*. Haemoglobin-content was measured with a hemaglobinometer†. In some cases thrombocytes were counted using a Coulter-platelet kit‡.

The breed, dosage regime, results of clinico-pathological investigations, examination of peripheral blood smears and the response to treatment are tabulated in Table 1.

OBSERVATIONS AND DISCUSSION

The wide range in oral dose rates (4,6 to 28,5 mg/kg) was somewhat unavoidable in the clinical situation be-

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† Department of Medicine, Faculty of Veterinary Science, University of Pretoria
§ Diff-Quick. Harleco
‡ Vibramycin 100 mg capsules. Pfizer Laboratories (Pty) Ltd. Jeppe Str. 259. Johannesburg.

§ Plasmalyte B., Sodium chloride 0,45 % (m/v) and Dextrose 2,5 % (m/v) injection BP. Baxter Laboratories, Inc, Deerfield, Illinois, USA.
‡ Phenamidine solution. Maybaker 21 McHardy Avenue, Port Elizabeth.
* Coulter-counter, Model FN. Coulter Electronics, INC. Hialeah, Florida, USA.
† Coulter Electronics INC. Hialeah, Florida, USA.
‡ Coulter Platelet Kit – Coulter Electronics Limited. Hertfordshire, England.

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TETRACYCLINES and TETRACYCLINES

Susceptibility of Common Pathogenic Bacteria to Seven Tetracycline Antibiotics in Vitro*

N H Steigbigel, C W Reed and M Finland

(From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Massachusetts)

Abstract: A total of 421 strains of various bacteria, nearly all of them recent isolates from infected patients, were tested for their in vitro susceptibility to seven tetracycline antibiotics by an inocula-replicating method and their relative activity against each category or organism was dipicted graphically. As judged by the proportion of strains against which each analogue was either the single most active one or was as active as any of the others, the order of activity of the seven analogues was: minocycline (67%), doxycycline (38%), methacycline (32%), chlortetracycline and demethylchlortetracycline (each 20%), and OTC and TC (each about 4%).

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Table 1: THE RESULTS OF TREATMENT OF EHRLICHIOSIS IN DOGS WITH DOXYCYCLINE

No. of case	Classification	Breed	Weight (kg)	Dose (mg/kg)	Duration of treatment (days)	Results of peripheral blood smear *E. canis*	*B. canis*	Hb g/ℓ	RCC 10^{12}/ℓ	Ht	WCC 10^9/ℓ	Thrombocyte 10^9/ℓ	Response to treatment
1	Acute	Dob.Pinscher	19,5	5,1	10 (oral)	pos.	pos.	34	1,26	0,11	11,7		Good
2	Acute	St. Bernhard	65	4,6	10 (oral)	pos.	pos.	146	5,83	0,45	7,7		Good
3	Acute	Alsatian	19	5,3	10 (oral)	pos.	neg.	94	3,82	0,30	9,2		Good
4	Acute	Schipperke	3,5	28,5	10 (oral)	pos.	pos.	80	3,09	0,26	8,2		Died
5	Acute	Cross-bred	17,5	5,7	10 (oral)	pos.	neg.	52	2,14	0,18	11,6		Good
6	Acute	Labrador	27	10	5 (i.v.)	pos.	pos.	50	1,92		68,1		Good
7	Acute	Fox Terrier	7,5	13,3	10 (oral)	pos.	pos.	35	1,75	0,14	6,2		Good
8	Acute	Poodle	14	13,4	10 (oral)	granules	neg.	86	3,46		9,2		Good
9	Acute	Alsatian	21,5	9,3	10 (oral)	pos.	pos.	39	1,9	0,17	15,1		Good
10	Biliary/ relapse	Cross-bred	6	16,7	10 (oral)	pos.	pos.	90	3,95	0,29			Good
11	Biliary/ relapse	Bull Terrier	12	5,3	10 (oral)	pos.	pos.	91	3,88	0,32	5,7		Good
12	Biliary/ relapse	Great Dane	46,5	10,7	8 (oral)	pos.	pos.	40	1,6	0,15	15,8	152,800	Died
13	Chronic	Irish Setter	26	7,7	10 (oral)	pos.	neg.						Good
14	Chronic	Cross-bred	17	11,7	10 (oral)	pos.	neg.	149	5,71	0,48	6,8		Good
15	Chronic	Alsatian	35	11,4	10 (oral)	pos.	neg.	108	4,5	0,36	5,4	110,500	Good
16	Chronic	Alsatian	21	9,5	10 (oral)	pos.	neg.	119	4,84	0,37	6,1		Good (relapse)
17	Chronic	Labrador	27	7,4	10 (oral)	pos.	neg.	100	4,7	0,36	11,3	85,650	Good
18	Chronic	Cross-bred	39	10,2	10 (oral)	neg.	neg.	136	6,13	0,41	4,3	73,000	Good
19	Chronic	Rottweiler	42	10	5 (i.v.)	pos.	neg.						Good
20	Chronic	Dalmation	23	8,6	10 (oral)	neg.	neg.	127	5,27	0,41	4,6		Good

Hb = Haemoglobin
Ht = Haematocrit
RCC = Red blood cell count
WCC = White blood cell count

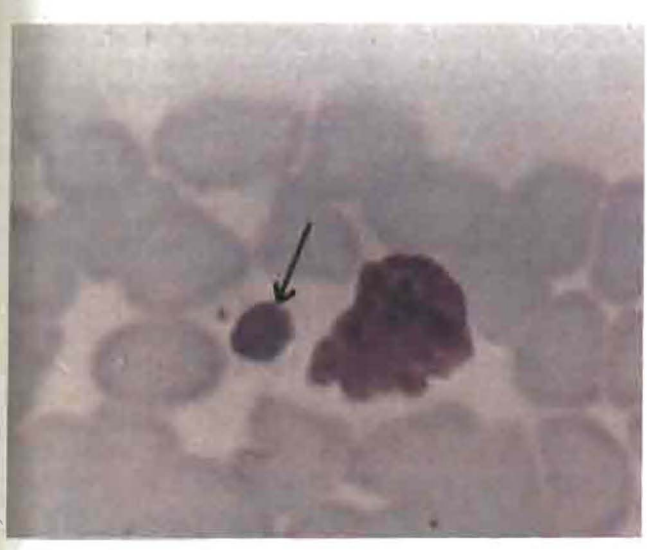

Fig. 1. Monocyte with morula of *E. canis* with its typical blueish internal granular structure in its cytoplasm.
Quik-stain x 2000

Fig. 2. Monocyte with both granules (a) and a morula (b) in its cytoplasm.
Quik-stain x 2000

cause only 100 mg capsules were available at the time of the trial.

Vomition occurred with the oral administration of doxycycline in two dogs (No 4 & 19). In both these cases vomition still occurred despite the tablets being given after a meal. The slow intravenous administration of the drug* was subsequently used in dog No. 19 and caused no problems. This route was also used successfully in dog No 6.

* Doxyvet injectable. Samvet, 20 Monument Street, Krugersdorp.

In spite of intensive treatment dog No 4 died. A post-mortem investigation revealed pathological changes suspicious for infectious canine hepititis.

Dog No 12 initially responded well to specific and supportive treatment but collapsed and died suddenly whilst being led from one kennel to another. A post-mortem investigation confirmed the diagnosis of ehrlichiosis and also demonstrated the presence of severe nephrosis.

The high incidence of a concomittant *Babesia canis* infection is to be noted. It is our opinion that ehrlichiosis is often overlooked because of the relative ease with

which *B. canis* trophozoites are demonstrated in peripheral blood smears. The finding of the latter in a peripheral bood smear usually terminates examination of the blood smear with the result that *E. canis* morulae are often overlooked. In practice all "biliary relapses" should be thoroughly investigated for ehrlichiosis. While the search for morulae of *E. canis* may be time-consuming, the finding of leucopaenia and thrombocytopaenia is together with anaemia indicative of canine ehrlichiosis.

All "Chronic" cases except No 14, were previously treated with oxytetracyclines. They were all presented for examination because of listlessness and a depressed appetite following an initial good response to treatment with oxytetracyclines. The period between last treatment with oxytetracycline and remission of symptoms varied from 21 to 42 days.

Dogs which are cleared of infection following treatment with oxytetracyclines are fully susceptible to reinfection with the homologous strain[2]. The possibility of reinfection in these cases cannot be excluded, however, it is thought that most of these cases were due to relapses. It is important to note that following treatment with doxycycline dogs returned to their original environment but did not relapse. (At the time of this report going to press the last patient had been treated at least three months previously.)

One noteable exception in the treatment response was dog No 15 (previously treated with oxytetracyclines) which was initially treated with doxycycline at a dose rate of 5,7 mg/kg. This dog relapsed after three weeks with fever, positive blood smear, listlessness, poor appetite and was subsequently treated with doxycycline at a higher dose rate of 11,4 mg/kg. It then made an uneventful recovery.

Dog No 18 was first treated at a dose rate of 5,1 mg/kg which was then raised to 10,2 mg/kg after 4 days because of the initial poor response to supportive and specific chemotherapy. It subsequently made an uneventful recovery.

Our findings indicate that an oral dose rate of 5 mg/kg once daily for 10 days is effective in the more acute cases of ehrlichiosis. In chronic cases an oral dose rate of 10 mg/kg once daily for ten days or 10 mg/kg intravenously once daily for five days is recommended.

The use of doxycycline in capsule form has an additional advantage over oxytetracycline in that the total dose is less bulky and thus more easily administered. Using oxytetracycline at an oral dose rate of 100 mg/kg for 10 days necessitates large quantities of capsules (250 mg) in large dogs which makes treatment by the owner impractical.

ACKNOWLEDGEMENTS

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BOOK REVIEW — BOEKRESENSIE

HEALTH ASPECTS OF HUMAN RIGHTS WITH SPECIAL REFERENCE TO DEVELOPMENTS IN BIOLOGY AND MEDICINE

WHO GENEVA 1976
(Van Schaiks Bookstore (Pty) Ltd., P.O. Box 742, Pretoria, 0001)
Price: Available on request from bookseller. pp. 48

This booklet does not contain information that is of immediate concern to veterinary science, except perhaps the section of two pages dealing with environmental protection. Nevertheless, to the veterinarian whom one assumes to occupy a position of responsibility in society, it makes interesting reading.

No hard and fast rules are laid down, but a wide variety of relevant topics listed below are discussed briefly in a general philosophical manner with references to detailed expositions.

Contents: Health as a human right, the beginning of life, artificial termination of pregnancy, newborn with congenital defects, use of human feotuses for research, sterilization, contraception, preventative medicine in genetic disorders, artificial insemination, human experimentation, tissue and organ transplants, computerized individual medical records, psychotherapy, environmental protection, compulsory measures for health protection.

THE TRANSMISSION OF CANINE EHRLICHIOSIS TO THE WILD DOG *LYCAON PICTUS* (TEMMINCK) AND BLACK-BACKED JACKAL *CANIS MESOMELAS* SCHREBER

J. VAN HEERDEN

ABSTRACT: Van Heerden, J.; **The transmission of canine ehrlichiosis to the Wild Dog *Lycaon pictus* (Temminck) and Black-backed Jackal *Canis mesomelas* Schreber.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 245-248. Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Canine ehrlichiosis was successfully transmitted from the domestic dog to three Wild Dogs *Lycaon pictus* and three Black-backed Jackals *Canis mesomelas*. Wild Dogs showed symptoms of anorexia and depression as well as anaemia, leucopaenia and mild thrombocytopaenia. Black-backed Jackals were asymptomatic. Morulae of *Ehrlichicia canis* were found in peripheral blood smears from all experimental animals. The disease was also successfully transmitted from Black-backed Jackal to the domestic dog.

INTRODUCTION

The wild dog *Lycaon pictus* is classified as a member of the family Canidae and belongs to the subfamily Cimocyoninae. This subfamily differs considerably from the other subfamilies of the Canidae[7].

Fluctuations in the numbers of wild dog *Lycaon pictus* have been ascribed to periodic disease outbreaks, and particular diseases affecting the domestic dog have been blamed. Rickettsiosis (ehrlichiosis) and canine distemper have been mentioned but positive confirmation of these infections is lacking[2 3 9 12 14].

The number of wild dogs in the Republic of South Africa are dwindling. This is probably because they are regarded as vermin in areas outside National Parks and are therefore ruthlessly destroyed[10 14].

Ehrlichiosis (Canine ehrlichiosis, Tropical canine Pancytopenia) is a disease of dogs caused by the rickettsial agent *Ehrlichia canis*. The brown dog tick *Rhipicephalus sanguineus* is an efficient vector of the rickettsial agent and infections tend to be persistent[5]. Inclusions of *E. canis* can be demonstrated in the cytoplasm of leukocytes in blood smears and also in mononuclear cells found in impression smears prepared from lung, spleen, liver and kidney of naturally and experimentally infected dogs[5].

Vertebrate hosts of *E. canis* are listed by Neitz as the domestic dog and the black-backed jackal *Canis mesomelas*[8]. The latter animal was infected by intravenous injection but did not develop any symptoms of disease. Subsequent subinoculations from this animal, however, showed it to harbour the parasite for a long period[8].

The coyote and the fox have also been successfully infected with *E. canis*[5]. Donation and Lestoquard's reported experimental infection in a monkey *Macacus inuus*, however, this work has not been repeated[5].

Pienaar[9] ascribed fluctuation in wild dog numbers to epizootics of rickettsiosis (ehrlichiosis) and referred to a publication by Stevenson-Hamilton[11]. Van der Merwe[12] referred to publications by Neitz and Thomas,[8] de Kock[3] and Stevenson-Hamilton,[11] and states rickettsiosis (ehrlichiosis) to be responsible for the death of a large number of wild dogs, hoewever, *E. canis* infection was not confirmed. Young[14] states the wild dog to be highly susceptible to *E. canis* but does not give the source of his information.

Stevenson-Hamilton[11] however did not report that any specific parasite was responsible for mortality but merely speculated on the possibility of a disease that could have caused deaths amongst wild dogs. The wild dog population decreased and ehrlichiosis was suspected but he never examined specimens or smears from either sick or dead wild dogs.

While Neitz and Thomas[8] had no opportunity to examine wild dogs or specimens from wild dogs, they did diagnose *E. canis* in a domestic dog in an area inhabited by wild dogs.

De Kock[3] made reference to the paper by Neitz and Thomas: "(they) recovered *Rickettsia canis* from some of the domestic dogs affected with the disease in the Kruger National Park and it is believed that the deaths in wild dogs may probably be associated with this parasite. It is quite likely that the disease is transmitted to dogs from a carnivore carrier such as the jackal which may not manifest symptoms."

The present study was undertaken to determine the susceptibility of wild dogs and black-backed jackals to experimental *E. canis* infection.

MATERIALS AND METHODS

E. canis

The isolate used in this experiment was obtained from a Bull Mastiff-cross from the Pretoria district, with severe ehrlichiosis and babesiosis. The dog was emaciated, depressed and anaemic with enlarged peripheral lymph nodes. A peripheral blood smear made in the way as described by Malherbe[6] and stained with Quickstain* was positive for both *Babesia canis* and *E. canis*.

Experimental animals

Three approximately 9 month old wild dogs were used in the experiment. A three month old domestic dog puppy (DI) served as a control. The wild dogs were reared in captivity and were identified by ear-tattooing (W1, W2 and W3). All these experimental animals were previously exposed to artificial *B. canis* infection by intravenous inoculation of *B. canis* infected blood 3 months previous to the trial. Blood from one of the wild dogs W3 was later transmitted to a 5-month old domestic dog pup (Dog O).

Three approximately 5-month old black-backed jackal pups reared in captivity (J2, J3 and J4) were used in the experiment. They had been previously exposed

* Diff-Quick Harleco.

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to artificial intravenous infection with *B. canis*. These animals were infected intravenously with *E. canis* and monitored as described for the wild dog.

Thirty-one days following the infection of J3 with *E. canis* 2 ml of heparinized blood was taken from J3 and injected intravenously into an approximately 5-month old domestic dog pup (Dog K).

Infection and monitoring of experimental animals

Blood was collected in a heparinized vacuum tube from the donor and 2 ml was immediately injected intravenously into each experimental animal. (W1, W2 and W3 and D1). Prior to the intravenous administration of infected donor blood haematological examinations were carried out and blood smears from all experimental animals were examined for the presence of parasites.

Each animal was examined daily by inspection for signs of disease. Blood was collected and blood smears were examined 13 times at relatively regular intervals during the ensuing 55 days. Blood was collected in sealed vacuum tubes containing EDTA. These samples were used for the determination of haemoglobin content, red cell count, packed cell volume, mean corpuscular cell volume, white cell count,* and thrombocyte count†.

Blood smears were made as described by Malherbe[6] stained with Quick-stain and examined under oil immersion for morulae of *E. canis* and *B. canis* trophozoites. The number of white cells containing morulae in 100 oil immersion fields were counted. Morulae of *E. canis* were thus recorded as the number of infected white cells per 100 oil immersion fields e.g. $^{5}/_{100}$; $^{10}/_{100}$.

Treatment of wild dogs and control pup D1

In order to prevent mortality from Day 35 onwards W1 was treated with tetracyclines§ and supportive fluid treatment.‡

W2 was treated on Days 42, 43 and 44 with 10 mg of tetracycline§ per kg body weight once daily parenterally. W3 was not treated. Control pup D1 was treated with a blood transfusion and tetracyclines.

Transmission from wild dog to domestic dog (Dog O)

Fifty-one days following the infection of wild dog (W3) with *E. canis*, 2 ml of heparinized blood was collected from W3 and injected intravenously into domestic dog pup (Dog O). This pup was temperatured daily and blood smears were examined at irregular intervals.

RESULTS

Infection in control domestic dog (D1)

The control domestic dog pup became mildly lethargic within five days following experimental infection. On Day 5 its temperature was 40°C and peripheral blood smears were positive for *Babesia canis* only. On Day 10 the puppy was clinically anaemic with enlarged peripheral lymph nodes and a temperature of 41°C. Blood smears were negative. On Day 11 peripheral blood smears showed the presence of morulae of *E. canis*. The smear was still positive for *E. canis* on Day 13. On Day 18 the puppy showed anorexia, weight loss, depression, anaemia, enlarged peripheral lymph nodes, a normal temperature and the presence of *E. canis* in monocytes in the blood smear.

The results of haematological studies, and examination of blood smears of D1 are listed in Table 1.

Table 1: HAEMATOLOGICAL INVESTIGATION OF CONTROL DOG D1 INFECTED WITH *E. CANIS*

Days:	1	3	5	7	10	11	13
Hb g/ℓ	124	125			62		47
RCC 10^{12}/ℓ	5,49	4,81			2,78		2,24
PCV	43,2	0,38			0,24		0,18
WCC 10^{9}/ℓ	7	5,8			5,3		4,2
Thrombocyte 10^{9}/ℓ	–	389,200			102,900		77,900
Smear	Neg.	Neg.	*B. canis*	Neg.	Neg.	$^{6}/_{100}$	$^{2}/_{100}$

Infection in wild dogs

The three wild dogs started to become listless from Day 27 onwards. They gradually became more and more inactive and spent more time sleeping and lying around.

W1 became completely anorexic on Day 34. On Day 35 it showed extreme listlessness and depression, total anorexia, mildly enlarged peripheral lymph nodes, diarrhoea and ulcers in the external nares. For the first time since they had been kept in captivity ticks were found on a wild dog. The ticks from W1 were identified as *Rhipicephalus evertsi*. On day 36 the diarrhoea became yellow and slimy and on the following day it turned profuse watery green. On Day 38 it developed into a red watery diarrhoea which persisted until Day 41 when it again turned greenish. During this period W1 although weak and amaciated was still aggressive and offered resistance whenever it was handled. Approaching W1 often induced vomition and on its release after being handled, it often assumed a squatting-position and showed tenesmus. All food except water (to which electrolytes had been added)* was refused during this period. Fresh warm vomitus was collected by using apomorphine tablets subconjunctivally on donor dogs as described elsewhere[13], and this was given to the sick pup. Following this treatment there was a gradual but steady increase in habitus and appetite. During this period W2 and W3 were noted on occasion to regurgitate in response to begging gestures from W1.

W2 and W3 became completely anorexic on day 37 for the ensuing six days. During this period only water was taken. Both dogs had slightly loose blackish faeces.

In order to establish the cause of the diarrhoea examinations was carried out for helminths, coccidia and viruses from the stools of the wild dogs all with negative results. The following bacteria were however isolated: *Enterobacter* sp.; *E. coli* 09:K35; *Streptococcus faecalis* and *Proteus* sp.

* Coulter Counter Model FN Coulter Electronics, INC. Hialeah Florida, USA.

† Coulter Platelet Kit – Coulter Electronics Ltd., Hertfordshire, England.

§ Engemycine – Gist-Brocades nv Delft Holland.

‡ Plasmalyte B., Sodium chloride 0,45 % (m/v) and Dextrose 2,5 % (m/v) injection BP. Baxter Laboratories, inc. Deerfield, Illinois, USA.

* Entersol Vetlab. Stephen Road, Ophirton, Johannesburg.

The results of repeated haematological investigations as well as examination of peripheral blood smears are shown in Table 2 and Figure 1. Morulae of *E canis* were found as early as Day 9 and as late as Day 42.

Table 2: HAEMATOLOGICAL VALUES FOR WILD DOGS OBTAINED ON DAY 0

Wild dog number	W1	W2	W3
Hb g/ℓ	172	171	166
RCC 10^{12}/ℓ	8,37	8,07	8,34
Ht	,52	,53	,53
WCC 10^9/ℓ	16,2	16,9	13,4
Thrombocyte 10^9/ℓ	–	–	–

The peak in the level of parasitaemia coincided with low white cell and thrombocyte counts as well as a low haemoglobin value.

In Fig. 1 the results of blood smears, white-cell counts and thrombocyte and haemoglobin-values were recorded as the percentage reduction of the valves obtained on day 0. For instance on day 40 e.g. the mean leucocyte count for the three dogs were $5.16 \times 10^9/\ell$ or 33.2 % of the value obtained on day 0.

The parasitaemia of *E. canis* was recorded in the same way except that each daily result was expressed as a percentage of the mean maximum parasitaemia (14 on day 28). The level of infection was thus expressed as a percentage simply to be able to compare it with haematological findings on one graph and does not reflect the percentage infected cells in the living animal. The values obtained on day 0 are shown in Table 2.

Infection in domestic dog (Dog O)

A fever reaction developed seven days following the intravenous inoculation of blood from Wild dog W3. Intracytoplasmic granules were demonstrated in a peripheral blood smear. The dog remained listless and anorexic for a couple of days and then made an uneventful recovery. At no time were *E. canis* morulae demonstrated.

Infection in C. mesomelas and attempted transmission to Dog K

The Black-backed Jackals showed no clinical symptoms of ehrlichiosis whatsoever although morulae of *E. canis* were detected in peripheral blood smears of all three animals fifteen days following artificial transmission. The only abnormal clinical pathological feature was a mild drop in red cell count and haemoglobin content.

Dog K developed a fever reaction seven days following the intravenous inoculation of blood from J3. Three days later the dog showed typical symptoms of canine ehrlichiosis and the following haemotological values were recorded: Hb 78 g/ℓ; Rcc $3,47 \times 10^{12}/\ell$; PCV 0,26 WCC $6.77 \times 10^9/\ell$. A peripheral blood smear demonstrated *E. canis* morulae and was rated $^5/_{100}$.

DISCUSSION

The extrapolation of diseases of the domestic dog to the Wild dog *Lycaon pictus* is not justifible as they are not that closely related species[7]. Many authors mentioned rickettsial outbreaks amongst free-living wild dogs without adequate confirmation[2 3 9 12 14].

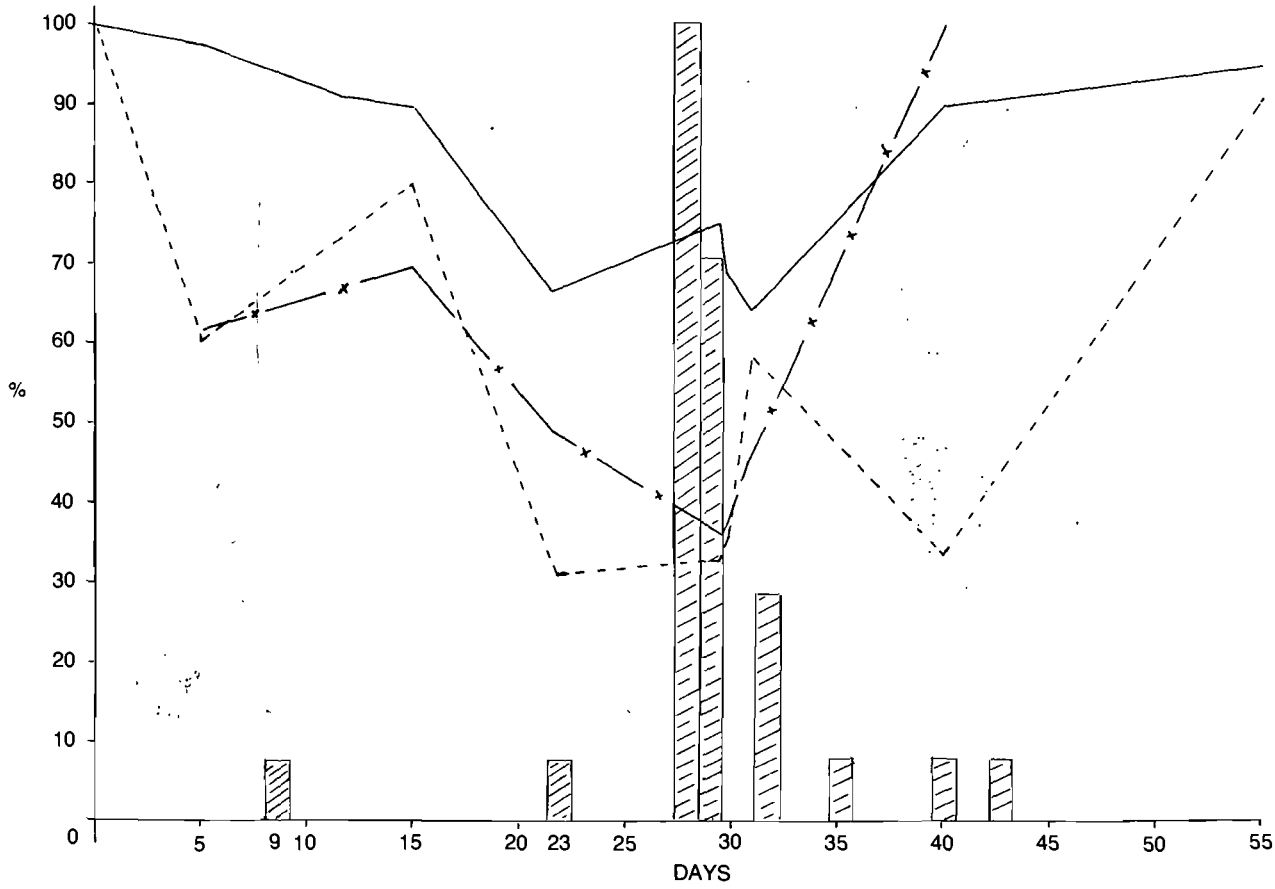

Fig. 1. Percentage haemoglobin, leucocyte and thrombocyte count in *E. canis* infected wild dogs – see text. – Hb; ---- Leucocyted; -x-x Thrombocytes; ▨ *E. canis* morulae.

In the present study the experimental transmission of *E. canis* to the wild dog caused symptoms of anorexia and depression as well as clinical pathological features typical of ehrlichiosis in the domestic dog. The leukopaenia, anaemia and thrombocytopaenia in the experimental animals was associated with the demonstration of *E, canis* morula in blood smears. In contrast to the average infection rate in the domestic dog, morulae in the wild dogs were more abundant (see Table 2).

In comparison to the control domestic dog, the incubation period in Wild Dogs was longer and symptoms of the disease were less severe. One wild dog W3 was not treated at all and another (W2) was treated for three days only.

In my experience domestic dogs should be treated orally for not less than ten days with oxytetracyclines in order to control the infection. Exact determination of the incubation period as determined by a rise in body temperature as the beginning of clinical signs, was impossible in the wild dogs as the rectal temperature in these animals was always elevated on handling.

The cause of diarrhoea towards the latter part of experimental infection is not known: Was this part of the symptomatology of *E. canis* in the wild dog, did it occur secondarily or was it perhaps the result of a superimposed infection? Diarrhoea is not a usual feature of ehrlichiosis in the domestic dog and it seems unlikely that it is a normal feature of the disease in *Lycaon pictus*. The frequent handling of the wild dogs could have built up to an extremely stressful situation whereby the natural defence mechanisms in the body were disturbed, resulting in diarrhoea. That the dogs were stressed was obvious from the fact that they sometimes vomited when they were approached and handling sometimes precipitated tenesmus.

Chronic diarrhoea is of some importance in captive wild dogs as it has been experienced in some other captive groups (Verster personal comm). The cause remains unknown but it seems likely that either viral infections (corona-virus, Parvo-virus) similar to that described in dogs[1] may be responsible or that a physiological stress-induced phenomenon may play a role. In the present instance however, only non-pathogenic bacterial agents were isolated from the faeces and viral investigations proved negative.

Two of the wild dogs were observed regurgitating to their diseased littermate W1. This might be of some importance in the survival of sick animals during natural infection.

The tick *Rhipicephalus sanguineus* has not been recorded as a normal parasite in the wild dog *Lycaon pictus*. Zoo records[4] of infestation do, however, exist. Wild dogs used in this experiment as well as other wild dogs handled by the author were relatively free of external parasites. Social "nibbling" between individuals or self-cleaning is probably an important factor in reducing the level of ecto-parasitism.

The black-backed jackal seems a likely reservoir host for *E. canis* infection since it is able to harbour and propagate the infection without itself becoming adversely affected. The infection was successfully transmitted from an infected jackal to the domestic dog. This is in accordance with the findings by Neitz & Thomas[8]. The Black-backed Jackal occurs widely in the Republic of South Africa and could therefore be an important local epidemiological factor in the spread and propagation of canine ehrlichiosis.

In conclusion the Wild Dog has been shown to be susceptible to *E. canis* and develops clinical signs (depression, anorexia and pancytopaenia) of Canine ehrlichiosis. It appears more resistant than the domestic dog with a longer incubation period, despite higher levels of parasitaemia. Clinical and haemotological findings were less severe and intensive treatment was not required. Whether the disease occurs naturally amongst free-living wild dogs is not known. The Black-backed Jackal develops a parasitaemia without clinical signs and could therefore act as a reservoir for infection. A serological survey for antibody-titres against *E. canis* in free-living Wild Dogs and Black-backed Jackals would perhaps yield more conclusive results.

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PARVOVIRUS AS A CAUSE OF ENTERITIS AND MYOCARDITIS IN PUPPIES*

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ABSTRACT: Van Rensburg I.B.J.; Botha W.S.; Lange A.L.; Williams M.C. **Parvovirus as a cause of enteritis and myocarditis in puppies.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 249-253 Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

A gastro-enteritis syndrome mimicking feline panleukopaenia was diagnosed in young dogs in the Republic of South Africa. Parvovirus was demonstrated by electron microscopy in the faeces of these animals. In addition an acutely fatal, acute to sub-acute non-purulent interstitial myocarditis occurred in pups in the same area. Histopathologically large basophilic intranuclear inclusion bodies were seen in the myocardium of these cases. The two syndromes generally occurred separately, but in two cases were found in the same individuals. The literature on the subject is briefly reviewed and the clinical and pathological findings in these outbreaks are reported.

INTRODUCTION

Canine parvovirus was first isolated from the faeces of healthy dogs in 1970 by Binn, Lazar, Eddy & Kajima[3]. Parvovirus was first associated with diarrhoea in puppies in Texas in 1977[8]. Eugster, Bendele & Jones in 1978 described a syndrome in dogs which very closely mimicked feline panleukopaenia and demonstrated parvovirus in the faeces of these cases by electron microscopy[7]. Within months similar outbreaks of infectious canine enteritis were reported from Canada[17], Australia[16] and Europe[16]. According to Pollock & Carmichael the parvovirus isolated from cases with gastro-enteritis appears to be distinct from the original isolate of Binn and his co-workers[16]. In a limited serological survey done by Black, Hoscher, Powell & Byerly[4] antibodies to the Binn isolate were quite common in banked serum samples collected before 1978 while antibodies to the pathogenic strain were only detected in sera collected after 1978.

In the past year reports have been published in various countries on the occurrence of acute deaths in puppies from a myocarditis of possible parvovirus origin[5 9 10 11 12 13 15 18]. The latter syndrome was characterised by a diffuse acute to sub-acute non-purulent interstitial myocarditis with inclusion bodies in the nuclei of myofibres. Electron microscopic examination revealed parvovirus-like particles in these inclusions[7 9 11]. Parvovirus has not yet been isolated from the myocardium, but recently Hayes, Russel & Babiuk demonstrated the presence of parvovirus in the inclusions by means of a fluorescent antibody technique[9].

In most outbreaks the myocarditis and gastro-enteritis syndromes have occurred as distinct entities. In one case, however, an overlap was reported in which both syndromes were manifested in the same patient[12].

Since the beginning of 1979 many similar outbreaks of either gastro-enteritis or myocarditis with acute death, have been diagnosed in the Pretoria-Witwaters-

Table 1: SUMMARY OF MAJOR FINDINGS IN SOME OF THE CASES EXAMINED

Case	Breed	Age	No. of pups dead/ No. of pups born	Main clinical signs	Pathology	
					Macroscopic	Microscopic
A	Alsatian	4–7 w.	6/6	anaemia, afebrile, acute death, leucopaenia.	anaemia, lung oedema	myocarditis with intranuclear inclusions. Lung oedema.
B	Pyrenian Mountain dog	4–6 w.	5/8	respiratory distress, tachycardia	anaemia, lung oedema catarrhal enteritis.	myocarditis with intranuclear inclusions. Lung oedema.
C	Boxer	± 5 w.	4/5	none observed	lung oedema, ascites, oedema of gall bladder wall	myocarditis with intranuclear inclusions. Lung oedema.
D	Labrador cross	10+ d.	6/7	diarrhoea, anaemia	lung oedema splenomegaly	myocarditis with intranuclear inclusions. Lung oedema, plus some intranuclear inclusions in intestinal epithelium
E	Rottweiler	6–8 w.	4/6	Vomition & diarrhoea	fibrinous enteritis	loss of epithelium, intranuclear inclusions
F	Afghan	6–7 w.	7/11	tachycardia	myocardial degeneration	Myocarditis with intranuclear inclusions
G	Ridgeback	12–14 w.	3/7	haemorrhagic diarrhoea, leucopaenia	focal erosive enteritis	dilation of crypts intranuclear inclusions

w. = weeks d. = days

* Paper read at the South African National and International Veterinary Congress, 3–7 September 1979, Johannesburg.

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rand area. The syndromes encountered locally were identical in all respects to those previously described. Attempts to isolate parvovirus from these cases have to date been unsuccessful, but further investigations are in progress. Electron microscopic examination of faeces from a puppy that died from gastro-enteritis revealed parvovirus particles, and histopathological examination of its intestine showed lesions typical of parvovirus enteritis according to the descriptions of Cooper, Carmichael, Appel & Greisen[6] and Kelly[14].

CLINICAL SIGNS

The details concerning age, breed, morbidity, mortality, main lesions, and other relevant particulars in the cases that we have encountered are given in Table 1.

In most of the outbreaks pups have showed evidence of either myocarditis or enteritis. Only in two outbreaks were enteritis and myocarditis encountered in the same individuals.

Puppies suffering from myocarditis very often died peracutely without any clinical signs having been noticed prior to death. In less acute cases the most constant symptoms were marked tachycardia (300 beats per minute) respiratory distress and anaemia. Some pups cried as if in pain and in one case the abdomen was distended. Diarrhoea may be present. The pups were afebrile and showed a moderate leukopaenia. Cases have been seen in pups from 10 days to 12 weeks of age – but most have been between 4 and 8 weeks of age. Electrocardiographic examinations revealed various abberations, especially arrhythmia and premature ventricular contractions of a low amplitude.

The outbreak in which parvovirus was demonstrated electron microscopically involved a litter of 6–8 weeks old Rottweilers. These pups showed anorexia, anaemia, moderate leukopaenia, vomition and diarrhoea. The latter varied from greenish to a black slimy stool. In some other cases the diarrhoea was more haemorrhagic.

PATHOLOGY

Macroscopic findings

Generalised anaemia and oedema together with focal areas of congestion of the lungs were the most constant findings in pups suffering from the myocarditis syndrome. In some cases oedema was generalised; anasarca, ascites, hydrothorax and hydropericardium being present. Perirenal oedema and marked oedema of the gall bladder wall were occasionally seen. The oedematous fluid was a watery slightly straw-coloured clear transudate with no fibrinous elements being present. Splenomegaly, and diffuse hepatic and apparent myocardial degeneration were frequently observed. In a few cases there was a catarrhal enteritis of the small intestine. One pup showed small focally disseminated haemorrhages in the lungs.

The majority of pups which had suffered from the principally gastro-enteritic form of the disease showed a mucocatarrhal to fibrinious pseudomembranous enteritis. The middle portion of the small intestine was particularly affected but in some animals the colon was also involved. One of the pups showed focal mucosal erosions in the small intestine. In others the small intestine was very congested and the contents were haemorrhagic. The myocardium may be of pale streaky appearance while in some the heart was enlarged due to ventricular dilation. A mild acute focal nephritis was encountered in one case while in another the gastric content was brown, haemorrhagic and mucoid.

Microscopical findings

Pups suffering from myocarditis showed a severe diffuse non-purulent interstitial myocarditis. There was a diffuse infiltration of lymphocytes, plasma cells and macrophages between the myocardial fibres which in focal areas aggregated into moderately dense masses which were frequently perivascular. There was a myolysis and disappearance of sarcoplasm from many affected fibres which resulted in a collapsed or deflated appearance of the sarcolemmal tube. Zenker's hyalin degeneration and calcification were not seen in any of these cases. Another constant and sometimes very conspicious finding in the haematoxylin and eosin stained sections was the presence of large basophilic intranuclear inclusion bodies in a varying percentage of myocardial nuclei. Nuclei containing these inclusion bodies were somewhat enlarged which facilitated their detection when examined under low magnification. The inclusions varied in appearance; the majority were large and cigar-shaped on longitudinal sectioning or round on transverse sectioning, stained very basophilically and were surrounded by a clear halo which resulted from a margination of the nuclear chromatin, while others were large, homogenous and blueish-grey, and completely filled the nucleus. They stained positively with Feulgen and Lendrum staining methods. Numerous Anitschkow myocytes were present in the areas of leukocytic aggregation and tubular collapse. In the more protracted cases there was some evidence of early fibroplasia of the myocardium.

Further there was a severe lung oedema and frequently some activation of alveolar macrophages. The Peyer's patches were prominent with large lymphoid follicles which, however, had a washed out appearance due to depletion of lymphocytes. In some cases the duodenal villi showed necrosis of the epithelium. The

Fig. 1. Loss of epithelium from crypts and villi in small intestine with mucosal collapse. Round cell infiltration into lamina propria.

Fig. 2. Dilated crypts filled with cellular debri and lined by flattened epithelium.

Fig. 3. Diffuse non-purulent interstitial myocarditis.

Fig. 4. Lymphocytic infiltration between myocardial fibres.

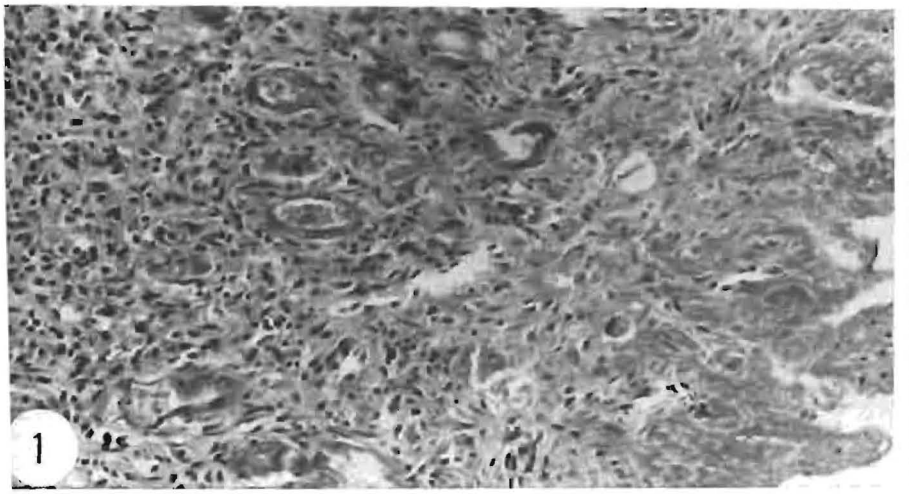
Fig. 5. Intranuclear inclusion body in intestinal epithelium lining crypt of Lieberkühn.

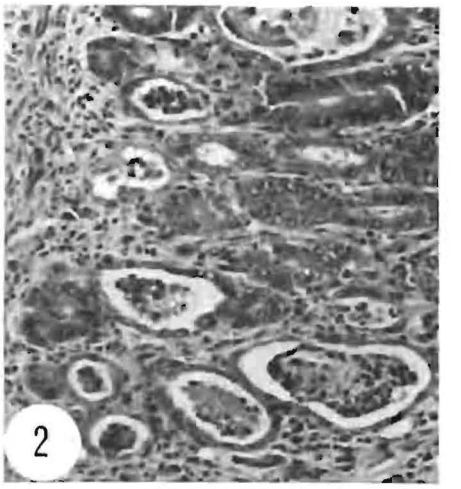
Fig. 6 & 7. Intranuclear inclusion bodies in myocardial nuclei surrounded by a halo due to margination of nuclear chromatin.

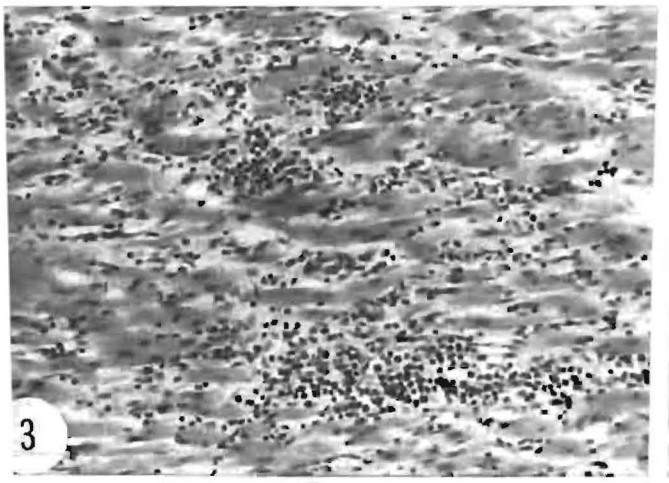
Fig. 8. same as above, but inclusion body completely filling the nucleus.

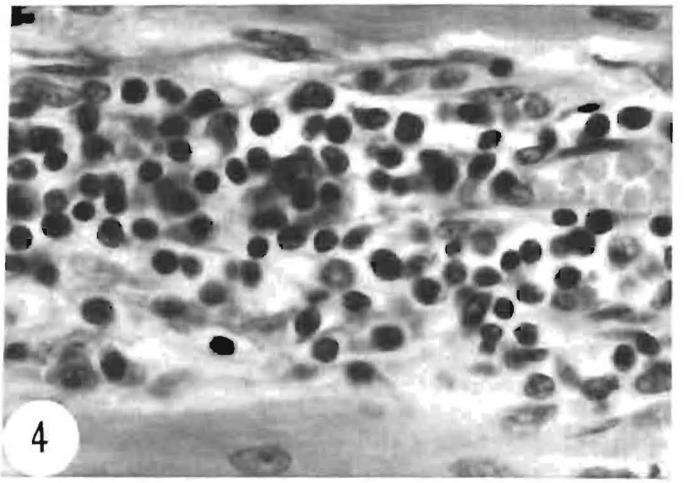
Fig. 9. Small intestine showing large dilated crypts.

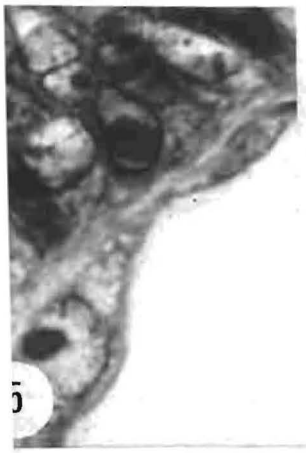
Fig. 10. Electron microscopic demonstration of parvovirus in dog faeces showing complete virons (white arrow) and empty capsids (black arrow).

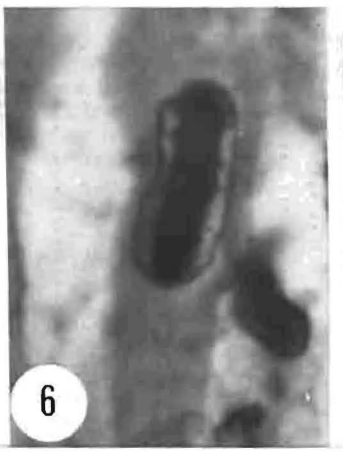


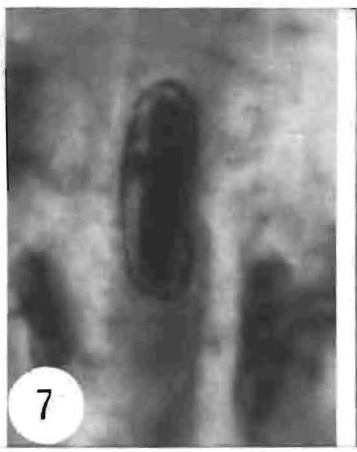


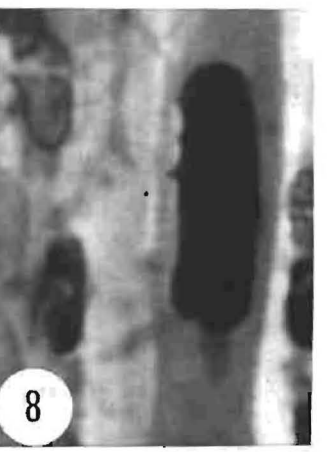


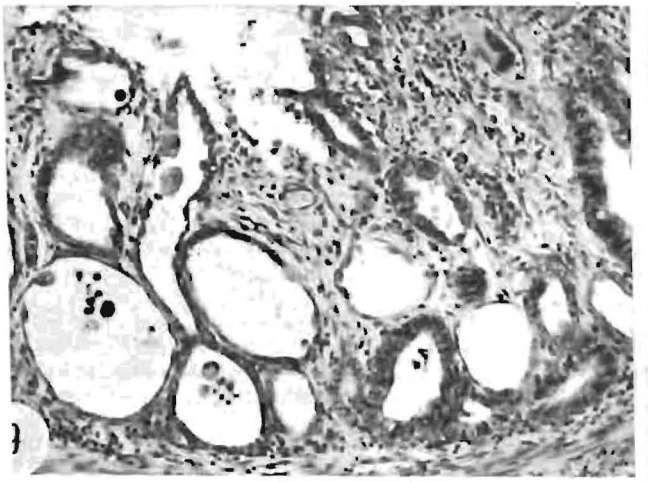


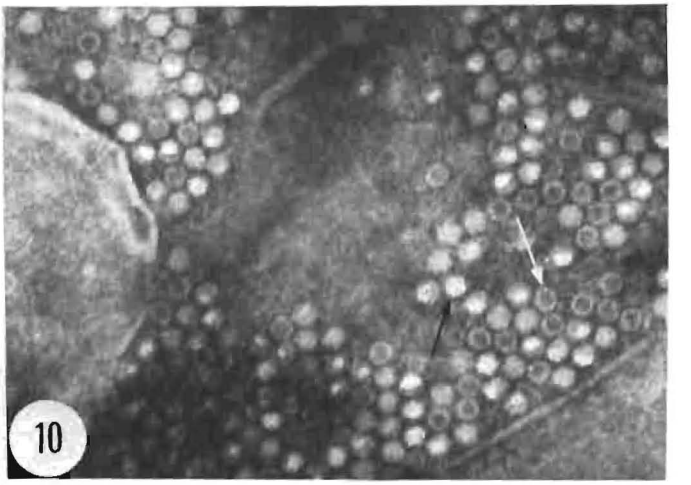












lamina propria was densely infiltrated by round cells and a few basophilic intranuclear inclusion bodies were seen in the epithelium near the necks of the glands of Lieberkühn in the ileum in two of the cases. Lesions in the spleen varied; some showed hyperplastic lymphoid follicles but in others the follicles had a "washed-out" appearance with necrosis of some of the remaining lymphocytes. Hydropic degeneration occurred in the liver while in one of the very young pups a perivascular oedema in the portal canals together with a mild leukocytic infiltration were present.

The cases suffering from gastro-enteritis did not reveal any histopathological evidence of a myocarditis. The main lesions were in the intestines where in some cases there was a marked denudation of the villi and crypts due to more or less complete loss of epithelium. This was accompanied by intense hyperaemia of the mucosa in some cases. In others many crypts were dilated and filled with cellular debri. Frequently such crypts were lined by a flattened attenuated epithelium. In some of the few remaining epithelial cells basophilic intranuclear inclusion bodies could be demonstrated. Bone marrow, thymus and lymphoid tissues were unfortunately not examined from these pups.

DISCUSSION

Parvovirus infection of dogs is a new disease which has not previously been recognised in South Africa. As yet the virus has not been isolated from any of these cases due probably to the fact that canine parvovirus is not easily isolated in tissue cultures. Binn *et al.*[3] could propagate it only in one out of 22 cell lines tested, while Black *et al.*[4] only managed to do this in the Crandill feline kidney cell line as the virus consistently failed to grow in cells of canine origin. However, the positive results obtained by electron microscopic examination, and the close similarity of the disease to that described recently in various overseas countries is strongly suggestive that the disease in South Africa is caused by canine parvovirus.

It is of interest that particularly the enteric form of the disease in dogs closely mimics feline panleukopaenia. Even more remarkable is the fact that the virus shares antigenic similarities, including neutralising antibodies, with feline panleukopaenia virus[4]. This, together with the fact that it preferentially grows on a feline kidney cell line, compels one to speculate that it could be a mutant of feline panleukopaenia virus. It is therefore not surprising that Pollock and Carmichael investigated the possibility as to whether or not feline panleukopaenia vaccines would protect dogs from parvovirus. They obtained favourable results using two doses of inactivated feline panleukopaenia vaccine administered at an interval of 1–2 weeks[16].

A characteristic of parvoviruses of practical significance in hospitals and clinics is that they are very resistant to inactivation. Sodium hypochlorite and formalin are the two agents of choice for disinfection[16]. Dog faeces are considered to be the primary source of infection.

The fact that parvovirus is associated with apparently two distinct syndromes, i.e. myocarditis and gastro-enteritis is suggestive that more than one strain of the virus might be involved. However, the fact that both syndromes are manifested in some patients, as well as the finding of Hayes *et al.*[9] that of three pups inoculated with a homogenate from affected myocardium, one developed parvoviral enteritis, strongly suggests that the same virus is indeed responsible for both syndromes.

As far as treatment is concerned no antiviral drug is available. Therapy is therefore symptomatic. Fluid and electrolyte losses should be countered by replacement therapy. Vomition and diarrhoea must be controlled and secondary bacterial complications eliminated. Keeping patients warm and minimising other stress conditions also seem to hasten recovery[16].

Viral diarrhoea in dogs may also be caused by coronavirus however the morbidity and mortality is significantly lower in this disease than is the case in canine parvovirus infection[1 2]. Furthermore in the disease caused by coronavirus there is no fever reaction or leukopaenia and pathologically much milder lesions with atrophy and fusion of the intestinal villi and a deepening of the crypts occurs. Other viruses which have been encountered in the diarrhoeic faeces of diseased dogs are rotavirus, paramyxo-like virus and adenovirus[16]. The significance of these is not fully known.

ACKNOWLEDGEMENTS

We wish to thank the various practitioners who submitted material and information to us, Prof. P.C. Howell for the virological examinations carried out, Dr A. Theodoridis for the electron microscopic examination of the faeces, Mr J. Soley and Mrs S.E. van der Hoven for photographic prints and Prof. R.C. Tustin for reading the manuscript.

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LETTER TO THE EDITOR — BRIEF AAN DIE REDAKSIE

MYOCARDITIS IN A PUP

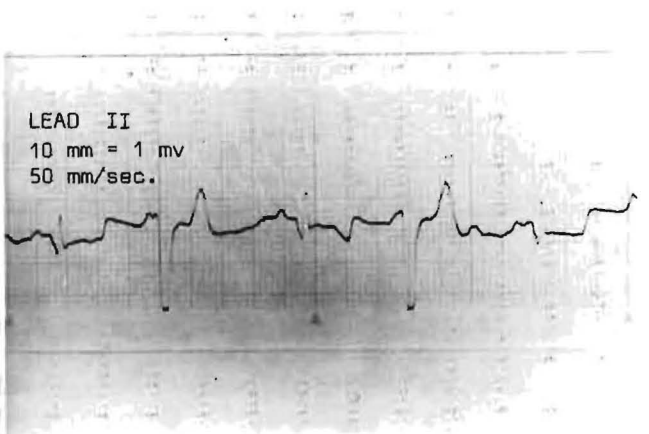
Sir,

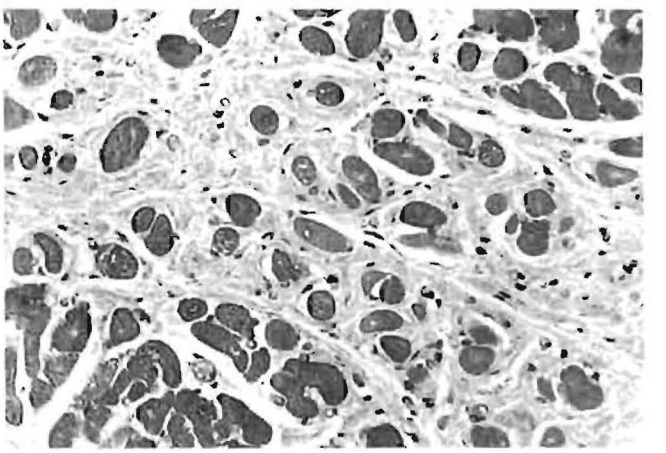
In May 1979 a four-month old Alsatian pup was presented in a state of severe respiratory distress. The only evidence of previous disease was an approximately 12-hour period of listlessness for 2 weeks prior to presentation.

A clinical examination revealed gross cardiac arrhythmia with severe pulse deficit and poor capillary filling time. A low-grade systolic murmur was detectable in the mitral valve area. Electrocardiographic examination showed multifocal ventricular beats and a tachycardia. The white cell count was found to be within normal range. Intravenous treatment with 2 % lignocaine brought immediate relief and this was followed by treatment with quinidine sulphate.

Four days later the dog was in a critical condition and once more sinus rhythm was restored with 2 % lignocaine.

An electrocardiogram taken two days later when the dog's habitus was excellent showed a tachy-arrythmia with ventricular bigeminy (Fig 1). Although the dog's habitus remained normal, it suddenly collapsed and died 48 hours later.





Post mortem examination showed pale white areas (approximate 1 cm diameter) in the left and right ventricular myocardium. Upon histopathology this proved to be focal areas of myocardial fibrosis. Individual myocardial fibres were atrophied within the fibrotic areas. The myocardial fibres were replaced by mature collagen which stained positive by the Von Gieson's and Masson's trichrome connective tissue staining methods (Fig 2). A mild perivascular and interstitial lymphocytic infiltration was also present. No inclusions were found within the myocardial cells.

Acute deaths amongst puppies with subacute non-suppurative myocarditis have been associated with viral aetiology. It is possible that the described lesions could have been caused by an earlier parvovirus infection.

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ANTHELMINTIC EFFICIENCY OF FENBENDAZOLE IN EQUINES

F.S. MALAN* and R.K. REINECKE†

ABSTRACT: Malan F.S.; Reinecke R.K. **Anthelmintic efficiency of fenbendazole in equines.** *Journal of the South African Veterinary Association* (1979) **50** No 4 255-258 (En) Hoechst Research Station, P.O. Box 124, 1320, Malelane, Republic of South Africa.

A single oral dose of fenbendazole (FBZ) at 10 mg/kg body mass was given to 5 donkeys. A further 5 donkeys were dosed with a medicated lick (1 mg FBZ/g lick) until the oral consumption was 10 mg/kg body mass.

In both trials FBZ was highly effective against adults of the following genera: *Cyathostomum, Cylicocyclus, Cylicostephanus, Cylicodontophorus, Poteriostomum, Cabellonema, Craterostomum* and *Triodontophorus;* similarly high efficiency was obtained against the following species: *Habronema majus, Habronema musca, Strongylus vulgaris* and *Oxyuris equi* and worms identified as belonging to the subfamily Cyathostominae.

These results were confirmed in horses and in addition FBZ at 10 mg/kg was highly effective against *Gyalocephalus capitatus, Oesophagodontos robustus* and *Parascaris equorum.*

INTRODUCTION

The efficiacy of fenbendazole (FBZ) for treatment of adult gastrointestinal nematode infections in horses has been demonstrated. At a dose level of 5 mg/kg FBZ is 100 % effective in the removal of adult *Strongylus vulgaris,* 99 % for adult *Strongylus edentatus,* 92 % for small strongyles, 100 % for mature *Oxyuris equi* and 80 % for mature *Parascaris equorum*[1]. Treatment of horses with FBZ at dose levels of 7,5–60 mg/kg produced negative faecal egg counts for a minimum period of 6 to 8 weeks[5].

This paper reports on critical anthelmintic tests in donkeys and modified critical anthelmintic tests on horses.

CRITICAL ANTHELMINTIC TESTS

MATERIALS AND METHODS

Two groups of 5 donkeys were kept on a cement floor each one in its own pen. Hay and water were supplied *ad libitum.* Body mass of donkeys was determined before treatment and worm egg counts done before and after treatment.

One group was treated with 10 % FBZ suspension* at a dosage rate of 10 mg/kg body mass *per os* with a stomach tube.

Another group was given FBZ mixed in a lick**. Each gram of the lick contained 1 mg FBZ and the total amount of FBZ consumed was 10 mg/kg body mass.

Every morning and afternoon for 7 days after treatment the total faecal output was collected from each donkey. Faecal worm egg counts were done every day.

Thereafter the faeces were broken between the fingers and mixed before determining the mass. In each specimen two aliquots of 1/10 and 1/100 by mass respectively were sieved with water on 150 μm sieves and the residue on the sieves surface collected and formalin added. Worms were counted macroscopically in the larger specimen (1/10) and microscopically in the smaller specimen (1/100).

The animals were slaughtered and methods used for worm recovery were the same as those previously described by Reinecke and le Roux[6].

Helminths were identified at a later stage by placing them on glass slides, clearing them with lactophenol, examining them microscopically and classifying them using the descriptions of Lichtenfels[3]. Cyathostomes were identified to genus level, but the species were determined in the other helminths.

RESULTS

Data are summarized in Tables 1 and 2.

The genera of Cyathostominae are presented in these tables under the heading of the subfamily. Details of the efficacy of FBZ at 10 mg/kg against the different genera of Cyathostominae are:-

1. *Cyathostomum, Cylicostephanus, Cylicodontophorus, Gyalocephalus, Poteriostomum* and *Cabellonema:* 100 %.
2. *Cylicocyclus:* In Group 1 it ranged from 93–100 % and in Group 2 from 99,9–100 %.
3. *Cylindropharynx* — ineffective.

The incidence of the various genera differed. *Cylicocyclus, Cyathostomum* and *Cylicostephanus* were always present and *Cylicocyclus* was the most prevalent in 8 of the 10 faecal specimens. *Cylicodontophorus* was less numerous and present in the faeces of 8 donkeys. *Poteriostomum* was present in two and *Gyalocephalus* and *Cabellonema* in the faeces of one donkey only. At necropsy, 8 *Cylindropharynx* were recovered from one donkey but none was present in the faeces. The efficiency against other adult strongyles varied as follows: *Triodontophorus, Craterostomum* and *Strongylus equinus:* 100 % and *Strongylus vulgaris:* 55,5–100 %.

Fenbendazole efficiency varied against adults of other species as follows: *Habronema majus:* 81,8–100 %, *Habronema musca:* 47,6–100 %, *Probstmayria vivipara:* 100 %, fourth stage larvae (L_4) and adult *Oxyuris equi:* 100 % but it was ineffective against adult *Trichostongylus axei,* L_4 and adult *Draschia megastoma,* 3rd instar larvae of *Gasterophilus nasalis* and *Gasterophilus intestinalis;* against L_4 of Cyathostominae its efficiency ranged from 19,2–88,1 %.

MODIFIED CRITICAL ANTHELMINTIC TEST IN HORSES

The term modified critical anthelmintic test is used to denote a combination of faecal worm egg counts and counts of worms expelled in the faeces. The host is not killed after treatment.

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* Panacur: Hoechst.
** National Chemical Products.

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Table 1: DONKEYS GIVEN A SINGLE DOSE FBZ AT 10 mg/kg LIVE MASS. WORMS RECOVERED FROM FAECES AND AT NECROPSY

Donkey Number	Site	Cyatho-stominae L_4	Cyatho-stominae Adult	*Triodontophorus*	*S. vulgaris*	*S. equinus*	*Craterostomum*	*H. majus* L_4	*H. majus* Adult	*H. musca*	*D. megastoma* L_4	*D. megastoma* Adult	*T. axei*	*P. vivipara*	*G. nasalis* L_3	*G. intestinalis* L_3
	Faeces	7 344	28 021	2 944	10	0	0	0	270	10	0	3	0	0	0	0
9034	Necropsy	2 208	188	0	8	0	0	0	60	11	4	77	0	0	31	4
	Reduction	76,9%	99,3%	100%	55,5%	–	–	–	81,8%	47,6%	0%	3,8%	–	–	0%	0%
	Faeces	1 100	11 992	200	10	10	10	0	10	0	0	0	0	36 200	0	0
0935	Necropsy	1 618	4	0	0	0	0	8	0	0	34	31	12	0	1	0
	Reduction	40,5%	99,9%	100%	100%	100%	100%	0%	100%	–	0%	0%	0%	100%	0%	–
	Faeces	13 262	110 482	360	10	0	2 164	0	130	0	0	0	0	0	0	0
0948	Necropsy	1 782	0	0	0	0	0	0	44	0	323	86	54	0	5	4
	Reduction	88,2%	100%	100%	100%	–	100%	–	74,7%	–	0%	0%	0%	–	0%	0%
	Faeces	9 709	29 242	300	380	0	3 462	0	150	10	0	0	0	0	0	0
0949	Necropsy	5 639[1]	74	0	0	0	0	0	0	0	0	0	0	0	12	0
	Reduction	63,3%	99,7%	100%	100%	–	100%	–	100%	100%	..	..	..	..	0%	–
	Faeces	1 706	725	20	120	0	12	0	70	0	0	0	8	0	0	0
0950	Necropsy	7 147[2]	28	0	10	0	0	0	10	0	122	84	20	0	0	2
	Reduction	19,2%	96,5%	100%	92,3%	–	100%	–	87,3%	–	0%	0%	28,6%	–	–	0%

[1] Plus 10 L_3 of Cyathostominae at necropsy
[2] Plus 138 L_3 of Cyathostominae at necropsy

Table 2: GROUP 2: DONKEYS HAD ACCESS TO A BLOCK CONTAINING 1mg FBZ/g AND WERE FED UNTIL THEY HAD CONSUMED 10 mg/kg LIVE MASS. WORMS RECOVERED FROM FAECES AND AT NECROPSY.

Donkey number	Site	Cyatho-stominae L_4	Cyatho-stominae Adult	*Triodontophorus*	*S. vulgaris*	*S. equinus*	*Craterostomum*	*H. majus* L_4	*H. majus* Adult	*H. musca*	*D. mega-stoma* L_4	*D. mega-stoma* Adult	*O. equi* L_4	*O. equi* Adult	*T. axei*	*P. vivipara*	*G. nasalis* L_3	*G. intestinalis* L_3
	Faeces	3 267	3 075	0	10	0	0	0	120	0	0	0	0	0	0	0	0	0
0943	Necropsy	8 144[1]	2	0	4	0	0	0	0	0	98	20	0	0	0	0	10	2
	Reduction	28,6%	99,9%	–	71,4%	–	–	–	100%	–	0%	0%	–	–	–	–	0%	0%
	Faeces	1 525	10 236	130	130	0	125	0	100	0	202	0	0	0	0	28 670	0	0
0944	Necropsy	3 386	0	0	0	0	0	0	1	0	87	3	0	0	76	0	0	0
	Reduction	31,1%	100%	100%	100%	–	100%	–	99%	–	69,8%	0%	–	–	0%	100%	–	–
	Faeces	6 949	7 860	240	40	0	11	0	40	10	0	1	230	10	0	0	0	0
0945	Necropsy	4 182[2]	0	0	0	0	0	0	0	0	140	41	0	0	16	0	0	1
	Reduction	62,4%	100%	100%	100%	–	100%	–	100%	100%	0%	2,4%	100%	100%	0%	–	–	0%
	Faeces	3 505	5 971	120	180	10	325	0	100	0	0	0	10	0	0	0	0	0
0946	Necropsy	5 095[3]	0	0	1	0	0	0	0	0	10	2	0	0	0	0	42	1
	Reduction	40,8%	100%	100%	99,4%	100%	100%	–	100%	–	0%	0%	100%	–	–	–	0%	0%
	Faeces	523	3 188	0	0	0	115	0	0	0	0	0	0	0	0	0	0	0
0953	Necropsy	214	0	0	0	0	0	1	0	0	141	1	0	0	66	0	5	0
	Reduction	71,4%	100%	–	–	–	100%	0%	–	–	0%	0%	–	–	0%	–	0%	–

[1] Plus 804 L_3 of Cyathostominae recovered at necropsy
[2] Plus 572 L_3 of Cyathostominae recovered at necropsy
[3] Plus 404 L_3 of Cyathostominae recovered at necropsy

MATERIALS AND METHODS

Seventeen horses from a Boerperd stud breeder were selected. Faecal worm egg counts were high and some had *Parascaris equorum* eggs.

The mass of each horse was determined and the animals were treated with a 10 % suspension of fenbendazole at a dosage rate of 10 mg/kg body mass administered through a stomach tube. The horses were individually kept in loose boxes and they were fed sugar cane tops *at libitum* and water was supplied.

Faeces were collected for a week every morning and afternoon. A 1/10 and 1/100 aliquot by mass of each sample was collected and washed on a 150 μm sieve. The residues were poured into glass jars and formalin was added.

Owning to the large numbers of worms the 1/10 aliquots were used and they were examined microscopically with a stereomicroscope.

The larger nematodes, i.e. *P. equorum* and *O. equi*, were removed from the total mass of faeces.

Egg counts and faecal cultures were made every 7 days for the three weeks following treatment.

RESULTS

Results of egg counts and worm recoveries are presented in Table 3.

The following genera and species were recovered from faeces after treatment.

Species	No. of horses infected
P. equorum	6
O. equi	14
S. equinus	3
S. edentatus	1
S. vulgaris	5
S. vulgaris L_4	12
Cyathostominae	13
Cylicocyclus	14
Cyathostomum	14
Cylicostephanus	14
Cylicodontophorus	10
Poteriostomum	3
Triodontophorus	3
Craterostomum	1
P. vivipara	7
Oesophagodontus robustus	3
Gyalocephalus capitatus	1

The worms were expelled within 5 days of treatment and eggs of both *P. equorum* and strongyles were absent in all the horses for at least three weeks after treatment. All the faecal cultures were negative for infective larvae.

In addition to the favourable results in the previous trials, FBZ at 10 mg/kg was highly effective against *P. equorum, Strongylus edentatus* and *Oesophagodontus robustus*. It is interesting to note that *O. equi* was present in faeces of 14 horses ranging in numbers from 5–4813 and 4940 *Gyalocephalus capitatus* were expelled in the faeces of one horse compared with a count of only 171 of this species in the faeces of a donkey.

DISCUSSION

These trials confirmed the results of other workers on adult strongyles[1,2]. We were able to confirm the results[2] on adult *H. majus* and *H. musca* but FBZ had no effect on adult or L_4 of *D. megastoma* nor L_4 of *H. majus*.

Most of the L_4 of Cyathostominae recovered at necropsy in our trials were present in the digested caecal and colonic wall and very few in the ingesta of these organs. Although critical anthelmintic tests were not a suitable method of assessing anthelmintic efficiency of either L_3 (third stage larvae) or L_4 of *Oesophagostomum columbianum* in sheep, because these larvae are digested in their passage down the intestinal tract[7], it would seem from the present results that this is not the case with L_4 of Cyathostominae in the caecum or colon of donkeys. Possibly the enzymes of the caecum and colon capable of digesting these worms are absent in equines accounting for their presence in faeces in a relatively undamaged state. Third stage larvae and L_4 of *O. columbianum* of sheep are either in the intestinal wall or L_4 in the lumen of the small intestine initially, before they migrate to the colon. Apparently enzyme action in the small intestine on dead L_4 of *O. columbianum* accounts for their absence in faeces after treatment.

Table 3: MODIFIED CRITICAL TEST IN HORSES. WORMS EXPELLED IN FAECES AFTER TREATMENT WITH A SINGLE DOSE OF FENBENDAZOLE AT 10 mg kg LIVE MASS

	Bruintjie	Breker	Brunet	Busuko	Calvyn	Ds Wit	Engela	Girlie	Hessie	Marissa	Mosjes	Nerine	Nettie	Pieter	Slpoor	Vossie	Vossies foal
	Worms expelled in faeces																
P. equorum	26	1	0	0	0	2	1	0	0	5	0	1	0	0	0	0	0
O. equi	381	15	1 023	0	0	2 353	2 376	4 813	5	501	3	0	1 761	5	498	11	37
S. equinus	0	0	10	0	0	0	0	0	0	0	0	0	18	0	0	11	0
S. edentatus	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. vulgaris	135	0	0	10	43	10	0	0	0	0	0	0	0	0	10	0	0
Triodontophorus	0	0	0	0	0	273	2 161	0	0	0	0	0	0	0	13	0	0
Craterostomum	0	0	0	0	0	0	0	0	0	0	210	0	0	0	0	0	0
L_4 of *H. musca*	0	0	0	0	0	0	0	0	0	0	0	200	0	0	0	0	0
P. vivipara	0	0	100	98	100	0	0	100	200	100	8	0	0	0	0	0	0
O. robustus	0	0	0	0	0	0	234	1	1	0	0	0	0	0	0	0	0
L_4 Cyathostominae	1 168	0	2 942	1	0	1 722	41 223	950	2 066	1 266	436	1 021	1 969	0	1 826	0	0
Adult Cyathostominae	37 882	0	31 780	92 141	127 997	28 161	47 556	67 667	219 847	40 603	12 484	25 679	72 543	0	25 269	79 478	0
	Faecal worm egg counts on day of treatment (Day 0) and every 7 days thereafter																
Day 0	2 800	66	4 300	3 000	1 200	2 267	2 000	2 000	1 800	1 400	2 000	1 100	2 900	0	3 133	2 067	333
Day + 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day + 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day + 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

We confirmed the observations of Duncan *et al*[2] on L_4 of Cyathostominae in the lumen and apparently showed that even at a dose of 10 mg/kg FBZ was 19,2–88 % effective against these larvae. This is an assumption only, because we had no method in a critical anthelmintic test of determining whether L_4 were present either in the ingesta or the caecal and colonic wall when the donkeys were treated with FBZ.

Subsequently, however, Malan[4] carried out a controlled anthelmintic test on donkeys. Most of the treated group were dosed with FBZ at 60 mg/kg while others were given higher doses from 120 mg/kg to a maximum of 800 mg/kg. He was unable to confirm the highly favourable results of FBZ at 60 mg/kg against L_4 in the intestinal wall[2] and even at higher doses the efficiency of FBZ varied.

In Malan's[4] and our trial aged donkeys were used while Duncan *et al*[2] worked with 6–12 month old ponies. Fenbendazole may not be as efficient in old donkeys as it is in young ponies.

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BOOK REVIEW — BOEKRESENSIE

VETERINARY HELMINTHOLOGY

ANGUS M DUNN 2nd Ed.
William Heinemann Medical Books Ltd London 1978
pp × + 323 Figs 153 Appendices 2 Publ Price £17,95

This book like the first edition is divided into three parts. Part I. The parasites is one of the best features of this publication and the illustrations are superb. The life-cycles are well described and up to date. Part II. The host-parasite relationship is only 8 pages long and so brief that comment is not warranted. Part III. The hosts. All the domestic animals and man are dealt with in separate chapters and in my opinion Dr Dunn has improved this part enormously if it is compared with the first edition. I still quibble with his lack of any firm commitment to anthelmintics but this is a minor point. Appendix I. Laboratory diagnostic aids has good keys for the diagnosis of infective larvae of ruminant and equine nematodes, but the section on post mortem examination is not of the same standard and should be revised and improved. Appendix II. This covers a wider field than is strictly necessary for undergraduate students but is very well presented.

As I said in my review of the first addition this is the best text book in English for undergraduate students, well written and presented and I recommend it for our colleagues interested in helminthology.

R.K.R.

DISSEMINATED INTRAVASCULAR COAGULATION: A REVIEW OF ITS PATHOGENESIS, MANIFESTATIONS AND TREATMENT

D.J. MOORE

ABSTRACT: Moore D.J. **Disseminated Intravascular Coagulation: A review of its pathogenesis, manifestations and treatment.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 259-264 (En) Orange Grove Veterinary Hospital, 119 Louis Botha Avenue, Orange Grove, Johannesburg 2192, Rep. of South Africa.

Disseminated intravascular coagulation is a clinicopathologic process of man and animals which occurs secondary to many diseases. The process may manifest in a variety of clinical syndromes including medical shock, haemorrhage, haemolysis and organ failure. A diagnosis can be confirmed by detecting a deficiency of several haemostatic components and the presence of raised levels of circulating fibrinogen (fibrin) degradation products. Therapy of the disorder includes the removal of the initiating factors which have provoked the clotting process together with the use of anticoagulants. The disease processes in which disseminated intravascular coagulation is implicated in the dog are enumerated.

INTRODUCTION

The term disseminated intravascular coagulation was derived by McKay[31] and is known by the acronym DIC and by various synonyms which include defibrinating syndrome, hypofibrinogenaemia, fibrination, diffuse intravascular thrombosis, thrombotic thrombocytopenic purpura, coagulation-fibrinolysis syndrome and generalized Shwartzman reaction[4 28].

DIC is defined as the pathological activation of the coagulation mechanism which leads to generalized intravascular clotting involving particularly the arterioles and capillaries and results in a consumption coagulopathy, intravascular haemolysis and a haemorrhagic diathesis[31 48 53]. The process can be acute, subacute or chronic and may be either localized or generalized[31 53]. DIC can be recognized by the appearance of medical shock, alterations in the levels of the circulating clotting factors, a haemorrhagic tendency and the presence of small vessel thrombi in a variety of vascular beds[31 53]. Stefanini[53] considers DIC to be an exaggeration of the normal mechanism of haemostasis.

MECHANISM OF COAGULATION

Stefanini[53] proposes that the physiologic mechanism of haemostasis has five integral components which are represented by: the vascular component, the platelets, the clotting mechanism, the fibrinolytic mechanism and the anticoagulant mechanism. Vascular integrity is preserved by the interaction of these components in the continual deposition and removal of fibrin along the vascular endothelium[31].

The Vascular Component

The intact vascular endothelium carries a negative charge which tends to prevent platelet adherence to the vascular wall[46]. In the event of vascular injury, this charge is lost which promotes platelet adherence[46]. Vascular injury results in an immediate vasoconstriction mediated via the axon reflex, which results in slowing of the blood flow in the injured area[53]. Vascular endothelial injury exposes collagen fibres to which platelets adhere, utilizing the enzyme glucosyl-transferase, located on the platelet surface[53]. Vascular wall damage results in the release of a haemolysin which haemolyzes nearby erythrocytes[40]. These cells in turn release erythrocyte thromboplastin and adenosine diphosphate which stimulates platelet aggregation and potentiates coagulation[40].

The Platelets

Platelets are essential for maintaining vascular integrity[12 47] and carry primary and activated clotting factors on their surface as well as the factors of the fibrinolytic system both on their surface and within[53]. The various platelet mediators which are active in coagulation include: platelet factor 1 which is a labile protein similar to factor V, platelet factor 2 which accelerates the conversion of fibrinogen by thrombin, platelet factor 3 which is a phospholipid essential to the intrinsic system of coagulation, platelet factor 4 which is a protein and has anti-heparin activity, 5-hydroxytryptamine, histamine, adrenalin, nor-adrenalin, thromboplastin, adenosine triphosphate, fibrin stabilizing factor and retractin[12 53]. The full activity of platelets in haemostasis depends on their adherence, followed by aggregation, viscous metamorphosis and clot retraction[53]. Platelet adhesion follows contact with thrombin or with exposed collagen fibres or foreign surfaces[53]. Platelet aggregation involves the conversion of platelet adenosine triphosphate to adenosine diphosphate and utilizes calcium ions and fibrinogen[53]. Viscous metamorphosis follows as the platelets swell and release various chemical mediators, of which serotonin, adrenalin and nor-adrenalin cause sustained vascular constriction at the site of injury and thus further slows the blood flow enabling the coagulation factors to reach maximal concentration and thus potentiate clotting[53]. Finally, once the stabilized fibrin clot is formed, retractin causes clot retraction[53].

The Clotting Mechanism

The clotting mechanism involves the activation of a series of clotting factors found in the plasma which constitutes two pathways of activity, namely the intrinsic and extrinsic systems[12 46 53]. The factors circulate in the inactive or proenzyme form and are activated by physical or chemical means, the activated factor then initiates the sequential reactions in a typical "cascade" or "waterfall" reaction[12 46 53]. The nomenclature of the coagulation factors has now been standardized by designating each with a Roman numeral (Table 1).

The intrinsic pathway (Fig 1) utilizes the constituents present in circulating plasma[12 46 47 53]. The first phase is the activation of factor XII by contact with vascular collagen, although procoagulants such as foreign surfaces, bacterial endotoxin and antibody-antigen complexes are also capable of activating this factor[12 53]. Activated factor XII converts factor XI to its active state

Table 1: NOMENCLATURE OF BLOOD CLOTTING FACTORS[a]

International nomenclature	Synonym
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Tissue Thromboplastin
Factor IV	Calcium
Factor V	Proaccelarin, labile factor, accelerator globulin, thrombogen
Factor VII	Proconvertin, Stable factor. serum prothrombin conversion accelerator, autoprothrombin I
Factor VIII	Antihaemophilic factor, antihaemophilic globulin, thrombo-plastinogen, platelet Cofactor I, plasma thromboplastic factor A
Factor IX	Plasma thromboplastin component, Christmas factor, platelet Cofactor II, autoprothrombin II, plasma thromboplastic factor B
Factor X	Stuart-Power factor
Factor XI	Plasma thromboplastin antecedent, antihaemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin stabilizing factor, Laki-Lorand factor, fibrinase

a Adapted from the recommendations of the International Committee for the Nomenclarue of Blood Clotting Factors (1962)

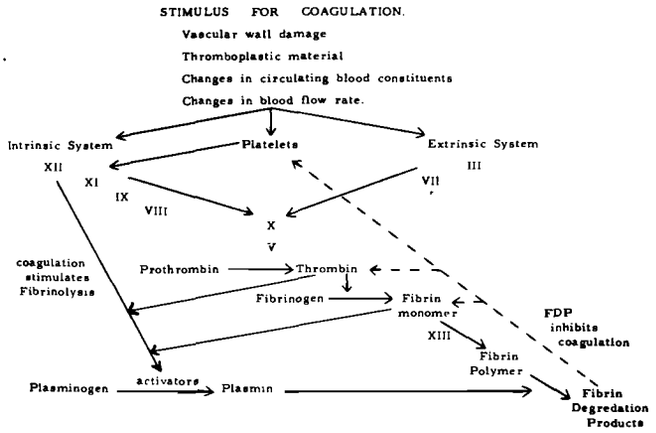

Fig. 1. A schematic summary of the pathogenesis of DIC.

as well as activating three proteolytic systems in plasma, namely kallikreinogen, plasminogen and complement which are capable of modifying inflammation and causing such unrelated symptoms as pain[5 12 53]. Activated factor XI in turn converts factor IX to its active state[12 46 53]. Activated factor IX in the presence of calcium ions activates factor VIII[12 46 53]. Activated factor VIII and platelet factor 3 in the presence of calcium ions form a complex which activates factor X[12 46 53]. Activated factor X and factor V complex with a phospholipid and catalyse the conversion of prothrombin to thrombin in the presence of calcium ions[12 46 53].

The extrinsic pathway (Fig 1) utilizes thromboplastin released from cells and tissues[12 46 53]. Factor III (tissue thromboplastin) in the presence of calcium ions complexes with factor VII and in the presence of more calcium ions, activates factor X which in turn converts prothrombin to thrombin in the presence of calcium ions, a phospholipid and factor V[12 46 53].

Thrombin acts on fibrinogen to form unstable fibrin monomer which is converted to stable fibrin polymer by the action of factor XIII, released from platelets, and which is itself activated by thrombin in the presence of calcium ions[12 46 53].

The Fibrinolytic System

Plasminogen (profibrinolysin), a plasma protein, is converted to the active state plasmin (fibrinolysin) by the release of activators from endothelial cells in contact with factor XII, thrombin and fibrin at the site of clot deposition[15]. Various other activators such as urokinase, streptokinase, lysosomal enzymes and trypsin can activate plasminogen[12 53].

Plasmin is an active protease which primarily digests fibrin and fibrinogen into small fragments but is also capable of digesting factors V and VIII[5 12 46 53]. The degradation products of fibrin and fibrinogen are indistinguishable and are referred to as fibrin-fibrinogen degradation products (FDP) or fibrin-fibrinogen split products (FSP) and designated fragment X, fragment Y, fragment D and fragment E in decreasing order of molecular weight[5 12 15 46 47 53]. These degradation products are rapidly removed from the circulation by the reticulo-endothelial (RE) system[12 53]. However, when they accumulate in high concentration in the circulation the larger X and Y fragments inhibit fibrin polymerization while the smaller D and E fragments inhibit thrombin, platelet aggregation and have hypotensive properties[5 12 15 53]. Thus, the fibrinolytic system (Fig 1) may significantly affect coagulation[12 15 53].

The Anticoagulant Mechanism

According to Stefanini[53] the continuous process of physiologic intravascular coagulation is effectively checked by the activity of anticoagulants which can be categorized into non-specific and specific anticoagulants.

The non-specific anticoagulants include the loss of thrombin through haemorrhage, the absorption of thrombin and other activated clotting agents onto plasma proteins, the vascular endothelium and the formed elements of the blood and the absorption of thrombin on the fibrin clot (antithrombin 1). The specific anticoagulants include antithrombin II (heparin Cofactor), antithrombin III, antithrombin IV (antithrombin III Cofactor), antithrombin VI (fibrin polymers) and heparin.

In addition, specific inhibitors are present for all the primary and activated factors of the process of blood clotting which emphasizes the concept of coagulation as a delicate balance between positive and negative forces of homeostatic coagulation[53].

PATHOGENESIS OF DIC

McKay[32 33] states that DIC is an intermediary mechanism of disease and that behind every pathological clotting episode lies an aetiological factor that triggers coagulation. The major categories of aetiological factors are: intravascular haemolysis, the release of thromboplastin, anoxia and anoxaemia, endothelial damage, bacterial endotoxin, proteolytic enzymes and particulate or colloidal matter[31 32 33]. These factors activate the intrinsic and/or extrinsic pathways of clotting to the degree that the normal homeostatic mechanisms are overwhelmed and DIC results (Fig. 1).

During the initial phase of coagulation the clotting factors are rapidly consumed and this is referred to as the hypercoagulable stage[31 53]. Thereafter follows a hypocoagulable stage when certain clotting factors, having been utilized, are depleted and a haemorrhagic diathesis results[31].

As a consequence of the excess clot formation and fibrin deposition, particularly in the microvasculature, the vascular lumen becomes obstructed. This causes physical injury and fragmentation to erythrocytes (schistocyte formation) which leads to haemolysis (microangiopathic haemolysis) and the release of erythrocyte thromboplastin[31 53]. The obstructed vascular lumen predisposes to hypoperfusion and tissue hypoxia which results in endothelial injury and stimulates the intrinsic clotting system[31]. Transudation of plasma from the injured vascular bed results in haemoconcentration and the agglutination of blood cells which aggravates the tissue hypoxia and results in tissue injury and the release of tissue thromboplastin, thus stimulating the extrinsic clotting system[31]. Excess fibrin formation potentiates the fibrinolytic system with resultant excessive FDP production which inhibits coagulation and aggravates the haemorrhagic tendency[5 12 15 31]. The RE system is responsible for rapidly clearing activated coagulation factors, procoagulants, fibrin, FDP and endotoxin from the circulation[5 7 12]. Shock and massive intravascular haemolysis result in RE system blockade which impairs this process and aggravates the severity of the coagulopathy[5 7 12].

CLINICAL MANIFESTATIONS

Although there are diverse aetiological factors leading to DIC, the functional and pathologic changes which follow such episodes are similar in many respects[31].

Hypotensive shock of varying severity is an almost universal component and is due to a decreased cardiac output resulting from a decreased venous return to the heart[17 31]. The latter is the result of obstruction to the blood flow through the hepatic and pulmonary circulation as a result of thromboses[17 31]. As a consequence, systemic hypotension exists together with pulmonary and portal hypertension[17 30 31 43].

As a result of the coagulation and haemorrhagic events the most common clinical symptoms include petechiae and ecchymoses of the skin and mucous membranes, haematemesis, haematochezia and sometimes gastrointestinal ulceration[15 31 53]. Neurologic manifestations include delirium, convulsion, stupor and coma with possible sequelae such as panhypopituitarism and diabetes insipidus[31 53]. Adrenocortical apoplexy with resultant adrenocortical insufficiency may occur[31]. Pulmonary insufficiency is manifested by dyspnoea, hyperpnoea and cyanosis[31]. Kidney involvement may be mild, resulting in oliguria and azotaemia, or severe, leading to acute renal failure, anuria, uraemia and death[31]. In some instances the nephrotic syndrome may supervene[31]. Pancreatic involvement can in some instances contribute to the patients demise[31]. Total occlusion of the circulation can lead to gangrene, often of the extremities, and is preceded by acrocyanosis[12 31].

McKay[31] concludes that DIC causes a wide spectrum of pathophysiologic changes. At one extreme the patient may die rapidly in profound shock, or the patient may survive the episode of shock and develop a haemorrhagic diathesis which may lead to death from massive haemorrhage. Alternatively, the patient may survive both episodes and develop a clinical disorder related to necrosis of certain organs. At the other extreme the patient may survive the clotting process totally unscathed.

CLINICO-PATHOLOGIC FINDINGS

Presumptive evidence of DIC can be obtained from peripheral blood smears which may reveal a responsive anaemia, spherocytosis, schistocytes, thrombocytopenia, leukocytosis or leukopenia[53].

Thrombocytopenia is usually present and sparse numbers of large abnormal forms may be present due to the consumption and rapid production of platelets[1 5 9 12 31 35 43 53 54 58].

There is usually a marked decrease in the coagulation factors with the concentration of fibrinogen, prothrombin and factors V and VIII being most severely depleted while factors VII, IX, X, XI and XII are less severely decreased[1 12 31 43 48 49 53 58]. The intrinsic clotting system is evaluated by means of the activated partial thromboplastin time (APTT) and the whole blood clotting time while the extrinsic system is evaluated via the prothrombin time (PT)[25 46 53]. The APTT and PT and whole blood clotting time may be shortened during the early hypercoagulable stage of DIC[17 31]. As a result of depletion of clotting factors the APTT, PT and whole blood clotting time are prolonged during the later hypocoagulable stage[12 31 53].

Increased levels of FDP are usually present and is an accurate method of determining the late phenomenon of excessive fibrinolytic activity in DIC[5 6 8 13 15 16 20 42 53 58]. Fibrinogen levels are usually decreased in DIC in man[1 5 12 31 53], while fibrinogen depletion is less frequently evident in DIC of dogs[6 15 23 29] as fibrinogen is readily produced in the dog's liver[15 16 54].

The dynamic nature of DIC contributes to the difficulty of diagnosis. This is particularly true in disorders associated with transient severe intravascular clotting leading to significant tissue damage, but in which depletion of the coagulation factors is also transient and may, in fact, be followed by supranormal levels[1 15 32 33 38 39].

ANATOMICO-PATHOLOGIC FINDINGS

Multiple haemorrhages are commonly disseminated over the entire body and involve the skin, mucosae, serosal surfaces and internal organs[16 31 35 53 58]. DIC is confirmed histologically by the presence of microthrombi which are usually present in the arterioles and capillaries and are associated with congestion, oedema and haemorrhage[31 48 53]. The microthrombi are of three types and are classified as fibrin thrombi, hyaline thrombi and platelet thrombi[48]. Fibrin thrombi are the most common and are best visualized histologically using the stains phosphotungstic acid haemotoxylin[48], or Martius-Scarlet-Blue (M C Williams 1977 Department of Pathology, Faculty of Veterinary Science, University of Pretoria, personal communication). In situ there is a rapid activation of fibrinolysis secondary to coagulation which occurs at a similar rate, whether or not the animal survives the episode of DIC[15]. Because most fibrin microthrombi are lysed within three hours of death[25], there is often a great variation between the gross and histologic findings in DIC[31 32 33 53]

TREATMENT

Because DIC is a fundamental pathogenetic mechanism in a wide variety of diseases, the best prevention and treatment lies in the eradication and cure of the underlying disease[15 31]. Treatment is further aimed at the removal of the contributary thromboplastic factors such

as haemoconcentration, metabolic acidosis, hypoxia, dehydration and haemolysis which cause further deposition of fibrin[15 53].

Fluid Therapy

Because hypotension is usually present in DIC[5 31] vigorous fluid therapy is essential in its management[5 26 32 45 53 56 60]. Although the choice of fluid is arbitrary[45 60] a balanced polyionic solution is preferred[45].

Colloids

The use of low molecular weight dextran has been advocated in the treatment of shock as it decreases the viscosity of blood, expands the plasma volume, opens the microcirculation and decreases platelet aggregation at a dose of 10 mℓ/kg body mass[15 32 42 45 53 56]. Caution must be exercised in the use of colloids in profound shock, sepsis or major burns as in these instances excessive capillary injury exists and escape of an osmotically active colloid into the interstitial space would alter the oncotic gradient and aggravate the interstitial oedema[45].

Anticoagulants

The paradox of treating a bleeding disorder with an anticoagulant necessitates the correct diagnosis in a suspected case of DIC[21]. The physiologic anticoagulant, heparin, is the drug of choice because it is immediately active and does not require an induction period as do the coumarin anticoagulants[34]. Heparin exerts its maximal anticoagulant effect by combining with antithrombin. The heparin-antithrombin complex is then able to inactivate various serine proteases such as plasmin and activated factors XII, XI, X, IX, VII and II[44]. Heparin also exerts anticomplementary properties, prevents immune haemolysis and inhibits platelet breakdown[10].

Heparin can be administered as an intravenous bolus at the dosage rate of 50 to 150 international units (100 i.u. = 1 mg) per kilogram body mass at intervals of 4 to 6 hours[1 15 32 33 43 56] or by continuous infusion[10 26]. Regardless of the method employed repeated monitoring of heparinization must be employed by maintaining the APTT or whole blood clotting time at 1,5 to 2 times normal[1 15]. Successful therapy results in the return of the coagulation factors to within normal limits[5 15 56].

Rapidly produced proteins such as fibrinogen and the coagulation factors respond within hours[1 15 30]. FDP are cleared from the circulation in 1 to 3 days and are thus less valuable in monitoring a response to heparin[5 15 42]. Platelet replacement requires longer than 3 days and thus their numbers are not an immediate determinant of successful anticoagulant therapy[5 15 42].

Heparin exerts its maximal pharmacotherapeutic effect during the initial hypercoagulable phase of DIC when it prevents thrombus formation[32 33] and in diseases which have a high prevalence of DIC heparin therapy should be instituted prophylactically[29].

Vasodilation

In order to alleviate capillary stasis and improve tissue perfusion in shock, vasodilation is occasionally of benefit in selected patients[15 57]. The most effective and safest vasodilator presently freely available is massive doses of glucocorticosteroids[37]. When methylprednisolone is administered as an intravenous bolus of 30 mg/kg body mass, the vasodilatory effect is sustained for about four hours following a single dose[37]. The glucocorticoids maintain the integrity of the microcirculation, stabilize lysosomal membranes and prevent the release of lysosomal proteases, decrease the adhesiveness and preserve platelet integrity, prevent the release of myocardial depressant factor, antagonize the action of endotoxin, have a positive inotropic myocardial effect, increase surfactant production, prevent degranulation of mast cells and increase lactic acid metabolism[37 52 53].

Controversy exists as to the use of the glucocorticoids in DIC at physiologic or pharmacologic dosage because they block the reticulo-endothelial system[1 7 15 17 53 55]. However, heparinization prior to or simultaneously with the use of the glucocorticosteroids negates this blockade[14]. Heparinization must thus precede or accompany glucocorticosteroid therapy in DIC[1 15].

Replacement of Depleted Coagulation Factors, Erythrocytes and Platelets

When severe haemorrhage is the major event following an episode of acute and extensive intravascular coagulation, replacement therapy with either whole blood, blood plasma or fibrinogen has been advocated to alleviate the anaemia, restore the platelet count and replace the clotting factors[1 5 15 32 33 43]. Because of the danger of providing substrate and aiding coagulation and thrombus formation during the early hypercoagulable phase of DIC it is essential to heparinize the patient prior to replacement therapy[1 15 32 33].

In DIC the generation of serine proteases such as thrombin and plasmin substantially reduces the plasma concentration of antithrombin[44]. Extensive lysis of platelets results in the release of platelet factor 4, which antagonizes the action of heparin[5 44]. Antithrombin is the essential cofactor required for heparin activity and some patients with DIC, refractory to heparin therapy, are often deficient in antithrombin[44]. Thus the replacement of depleted coagulation factors, in particular antithrombin, may be mandatory for the successful treatment of DIC with heparin[44].

Individual Organ Support

It is important to give supportive therapy to any organ system adversely affected by the process of DIC[15 53]. Mannitol or other diuretics are administered to reduce cerebral and pulmonary oedema and to prevent acute renal failure[15 56].

Fibrinolytic Activators

Streptokinase and urokinase are agents which activate the proteolytic enzyme plasmin and thus dissolve fibrin and fibrinogen but do not remove preformed thrombi[1 32 33]. Their activity may be useful while extensive coagulation is an ongoing process, however, the risk of artificial fibrinolysis producing a haemorrhagic diathesis precludes their use in the treatment of DIC[32 33].

Fibrinolytic Inhibitors

Epsilon amino caproic acid and Aprotinin* are agents which inhibit the fibrinolytic enzyme system[1 32 33]. These agents can be useful when fibrinolytic activity becomes a predominant problem and continues to the point that the coagulation factors remain at low levels because of the continuous activity of fibrinolysis[32 33]. Fibrinolytic inhibitors should not be used during the active coagulation phase of DIC since they would promote thrombus formation[2 15 32 33 43 53].

REPORTED CASES OF SPONTANEOUS DIC AND FIBRINOLYSIS IN THE DOG

A variety of clinical disorders which may be associated with intravascular coagulation and fibrinolysis have been described in the dog. These include thyroid carcinoma[49], mammary carcinoma[50], lymphatic leukaemia[51], myeloproliferative disease[22], haemangiosarcoma[24], haemothorax and haemangiosarcoma[27], shock[48 51], congestive heart failure[51], heartworm disease[23], suppurative bronchopneumonia[24], bronchopneumonia and hepatic necrosis[24], hepatic necrosis[54], diaphragmatic hernia[11], gastric dilatation-volvulus[26 59], haemorrhagic gastroenteritis[3], heatstroke[51], post surgically[46], haematoma of the stifle[21], viper bite[19], infectious canine hepatitis[58], leptospirosis[24], aflatoxicosis[16] and *Babesia canis* infection[35 36].

This paper is presented to provide a general review of the process of DIC and to enumerate the diverse clinical disorders in which DIC is implicated in the dog. It is hoped that more widespread recognition of DIC will lead to improved methods of early diagnosis and treatment.

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* Trasylol-Bayer.

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BOEKRESENSIE

BOOK REVIEW

DIE ULTRASTRUKTUUR VAN DIE SEL

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met Tegniese hulp van S. KEMPFF en N.V.D.W. LIEBENBERG,
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Butterworth & Kie (SA) (Edms) Bpk, 1979, Galestraat 152-154, Durban 4001
bl. 243; Hoofstukke 9; 149 mikrofotos en 17 sketse. Prys R22,50.

Hierdie boek is die eerste wat in Afrikaans verskyn op die gebied van normale ultrasitologie. Dit is werklik 'n voortreflike werk wat die sel met sy plasmalemma, alle organelle en kern, beide morfologies en funksioneel in redelike diepte bespreek. Daarby is dit met 'n magdom oorspronklike mikrofotos en diagrammatiese sketse ryklik geïllustreer. Die mikrofotos (die meeste is transmissie-elektronmikrofotos) is almal van 'n besondere hoë kwaliteit en is dus ideaal geskik vir die navorser wat nog probleme ondervind met die interpretasie van ultrastruktuur. Nie alleen word die organelle elk in 'n eie hoofstuk bespreek nie, maar normale variasies wat hulle in verskillende selle mag ondergaan word ook genoem. Dit is juis laasgenoemde aspek wat hierdie boek by die interpretasie van ultrastruktuur van groot waarde sal maak.

W.H.G.

DISSEMINATED INTRAVASCULAR COAGULATION: A COMPLICATION OF *BABESIA CANIS* INFECTION IN THE DOG†

D.J. MOORE* and M.C. WILLIAMS**

ABSTRACT: Moore D.J.; Williams M.C. **Disseminated intravascular coagulation: a complication of *Babesia canis* infection in the dog.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 265-275 (En). Orange Grove Veterinary Hospital, 119 Louis Botha Avenue, Orange Grove, Johannesburg 2192, Rep. of South Africa.

Disseminated intravascular coagulation is described as a complication of *Babesia canis* infection in the dog. *B. canis* infection in the dog is characterized as a mild (uncomplicated) or severe (complicated) disease. The clinical, coagulation and haematological, pathological and histopathological findings of the severe disease are described. Thrombocytopenia is reported as occurring in both the mild and severe forms of *B. canis* infection in the dog.

INTRODUCTION

Babesia canis is a tick-transmitted haemoprotozoon first recognized as a cause of disease in the dog in Italy[37]. The disease was first encountered in the Cape Colony by Hutcheon[21] in 1885 but he failed to recognize the causative organism. *B. canis* infection in the dog is almost world-wide in distribution, occurring particularly in tropical, subtropical and temperate zones[28]. In the Republic of South Africa the disease is holoendemic over the greater part of the country[28].

The typical disease in the dog has been well documented[10 15 19 21 25 27 28 29 43 48]. Biliary fever in the dog in the Republic of South Africa is a hyperacute, acute, subacute or chronic, febrile, noncontagious, infectious disease[19 28]. According to Malherbe[27] biliary fever is typically characterized by depression, malaise, anorexia, progressive anaemia, rapid bounding "water hammer" pulse, splenomegaly, prostration and death. Constipation, excessive bile pigmentation of the faeces, haemoglobinaemia, haemoglobinuria and icterus may occur[27]. In chronic infections there is usually an intermittent fever, the appetite is capricious and the loss of condition marked[27].

Babesiosis in the dog has been described by Malherbe and Parkin[26] who aptly state that "the atypical forms of the disease may masquerade in such a variety of guises that one is left with little doubt that many cases of babesiosis are not diagnosed." The atypical manifestations of the disease include ulcerative stomatitis[26], gastrointestinal disturbances[26 27], nervous symptoms[2 5 26 27 38 39], muscular aberrations[2 26], respiratory symptoms[26 27] and circulatory disturbances[26 27].

The analogy between the manifestations of atypical *B. canis* infections in dogs and the complications of *Plasmodium falciparum* malaria in man has been drawn by several authors[2 5 25 26 27 39]. The pathogenesis of severe, complicated malaria with cerebral manifestations remained obscure until 1966 when Devakul, Harinasuta and Reid[9] incriminated disseminated intravascular coagulation (DIC).

The term DIC was derived by McKay[32] and is defined as the pathological activation of the blood coagulation mechanism leading to widespread intravascular clotting involving particularly the arterioles and capillaries, and resulting in a consumption coagulopathy, microangiopathic haemolysis and a haemorrhagic diathesis[32 33 45 47]. The process can be acute, subacute or chronic, localized or diffuse and is often the fundamental pathogenetic mechanism in a wide variety of disease processes[32]. DIC may be recognized by the appearance of shock, alterations in the levels of the circulating clotting factors, a haemorrhagic tendency and the presence of small blood vessel thrombi in a variety of vascular beds[32].

DIC has been reported in splenectomized calves with *Babesia argentina* infection[8] but has not been reported in dogs with *B. canis* infection. This paper describes a detailed examination of 12 dogs with canine babesiosis, 6 with mild and 6 with severe disease and reports the findings of DIC in dogs with severe disease.

MATERIALS AND METHODS

Twelve randomly selected naturally infected clinical cases of canine babesiosis which were presented for diagnosis and treatment were studied.

For convenience in recording and assessing the results of determinations within the confines of a progressive disease, the affected animals were arbitrarily placed into two clinical categories roughly reflecting the progression of the disease. These categories could from their nature, not be clearly delineated, as, inevitably, cases occurred which were borderline and had to be placed in one or other category where they best conformed at the time of presentation.

The criteria used in the clinical evaluation of the two categories were as follows:-

1. Mild (uncomplicated) infection – anorexia or capricious appetite, lethargy, mucous membranes pink to white, normal or bounding pulse, no macroscopic haemoglobinuria although traces (1+) haemoglobinuria may infrequently be present.
2. Severe (complicated) infection – anorexia, depression, mucous membranes red to white, bounding or rapid weak pulse, often macroscopic haemoglobinuria, usually traces (4 to 5+) haemoglobinuria. Any one or more of the following symptoms would automatically constitute severe disease: macroscopic haemoglobinaemia, haemorrhages on any mucous membrane or skin, nervous symptoms, vomition, diarrhoea, angioneurotic oedema, dyspnoea, cyanosis, epistaxis or icterus.

A definite clinical diagnosis of *B. canis* infection was made in each patient by examining a thin capillary

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blood smear prepared by stylet puncture of the ventral surface of the ear. Blood smears were made according to standard technique[28], air dried, fixed with absolute methyl alcohol and stained for 30 seconds in Stevenel's Blue Stain[17]. The excess stain was washed off the slide with slowly flowing tap water, dried and examined microscopically under oil immersion.

In each patient blood collection was made from the external jugular vein before treatment was instituted. For coagulation studies, the blood was collected in polypropylene disposable syringes and placed in 3,8 % trisodium citrate in the proportion of 9:1. Whole blood samples were collected into evacuated blood collecting tubes* containing EDTA. Blood for fibrinogen degradation product (FDP) analysis was collected into evacuated tubes containing a trypsin inhibitor and thrombin**.

Coagulation studies included the prothrombin time (PT) which was estimated according to Biggs[3]. The activated partial thromboplastin time (APTT) was determined using the activated thrombofax reagent†. Plasma fibrinogen (Fib.) was determined by the method of Ellis and Stransky[11], while the fibrinogen degradation products (FDP) were estimated semi-quantatively using a latex suspension‡.

Haematological studies included the leukocyte count (WCC), erythrocyte count (RCC), haemoglobin concentration (Hb) and packed cell volume (PCV) which were determined by electronic counter. The platelet count (Plat.) was determined manually by utilizing a standard technique[3]. The differential leukocyte count was determined by a standard technique[44]. Haemoglobinaemia was estimated macroscopically by examining the plasma of each patient for a pink discolouration. The plasma was obtained utilizing a microhaematocrit technique[44].

Urine analysis was performed macroscopically using a colourimetric multistrip test* which determined the presence of nitrite; pH range between 5 and 9; protein estimation determined as 1+, 2+ or 3+ corresponding to 30, 100 or 500 mg/dℓ; glucose as 1+, 2+ or 3+ corresponding to 100, 300 or 1000 mg/dℓ; ketones as 1+, 2+ or 3+; urobilinogen as 1+, 2+ 3+ or 4+ corresponding to 1, 4, 8 or 12 mg/dℓ; bilirubin as 1+, 2+ or 3+; blood/haemoglobin as 1+, 2+, 3+, 4+ or 5+.

The urine specimen was centrifuged at 1000 rpm for 10 minutes in order to obtain a sediment. The supernatant was decanted and the sediment stained** and examined microscopically. The supernatant was used for specific gravity determination utilizing a Goldberg refractometer†.

Autopsies were performed on two cases within two hours of death. The entire brain and various tissue specimens were collected and fixed in 10 % neutral formalin. Serial coronal sections of the brain approximately 3 mm in thickness were prepared and studied for lesions. Suitable areas from these slices and blocks from other tissues were cut and processed in an autotechnicon. Sections 3μm in thickness were cut and stained with haematoxylin and eosin, as well as Martius-Scarlet-Blue according to standard procedure[13]. Martius-Scarlet-Blue stains nuclei blue-black, erythrocytes yellow and fibrin red[13].

* Venoject-Terumo.
** Thrombo-Wellcotest sample collection tubes, Wellcome Laboratories.
† Ortho diagnostics, distributed by Ethnor.
‡ Thrombo-Wellcotest, Wellcome Laboratories.
* Combur 8 Test – Boehringer.
** Sedistain – Clay Adams.
† American Optical Company.

RESULTS

Twelve dogs of various breeds were studied, six of whom had mild disease while the remainder had severe disease. The sex distribution was 8 entire males, 2 entire females and 2 spayed females. The average age of the subjects was 44 months, but ranged from 4 months to 14 years. The average age of the group exhibiting mild disease was 43,5 months, while the average age of the group with severe disease was 44,5 months.

Clinical Findings

The significant clinical features of the patients at presentation are summarized for the mild category in Table 1 and for the severe category in Table 2. The initial symptoms observed by the owner in each instance were lethargy/depression and/or anorexia, except in case 6 where the dog had a capricious appetite and lost weight. The duration of symptoms was the approximate time which elapsed between the initial symptoms observed by the owner and the time of presentation. The mean duration was 82 (range 12 to 336) hours for the mild category and 30,5 (range 12 to 72) hours for the severe category. All the dogs were pyrexic at presentation with a mean rectal temperature of 40,0°C (range 39,2 to 40,7°C). Mucosal colour in the mild group varied from pink to light pink while that in the severe group varied from dark red to pink to white.

Haemorrhages occurred on the mucosae of 3 of the 6 dogs with severe disease (Cases 7, 8 and 9). Angioneurotic oedema was present in Case 10.

None of the cases in the mild group had abnormalities of the central nervous system (CNS) while 5 of the 6 cases in the severe group had marked depression of the CNS as exhibited by coma (Case 7) or stupor (Cases 8, 10, 11 and 12) and in which the spinal and postural reflexes were either depressed or absent. A state of catalepsy was present in Case 11. Chemosis was present in Cases 7 and 11 while Case 7 also exhibited nystagmus and Case 11 anisocoria.

A bounding ("water hammer") pulse was present in 7 of the 12 dogs (Cases 2, 3, 4, 5, 6, 9 and 12). A rapid weak pulse was present only in patients of the severe category (Cases 7, 8, 10 and 11). A normal pulse was present in Case 1. Splenomegaly was detectable in Cases 3, 6, 9, 10, 11 and 12. Pain on abdominal palpation was exhibited by case 11. Dehydration (Case 7, 8 and 11), dyspnoea (Cases 7, 8, 10 and 12), hypersalivation (Case 12) and vomition and/or diarrhoea (Cases 7, 11 and 12) were observed only in the severe category.

Urine examination

The findings of the urine examination are presented in tabular form in Table 3. Eight of the ten urine specimens examined (Cases 1, 3, 4, 6, 7, 8, 9, 10, 11 and 12) revealed a degree of renal injury, assessed as cellular casts, granular casts, renal tubular epithelium and pigment, regardless of the category of dog affected.

Table 1: SIGNIFICANT CLINICAL FEATURES OF MILD CATEGORY

Case No.	Breed	Age	Sex	Duration of Symptoms	Rectal temperature	Clinical Findings
1	Pomeranian	12 mth	M	48 hr	40 °C	listless, anorexia, mucosae pink, pulse normal.
2	Alsatian	30 mth	Fsp	12 hr	40,2 °C	listless, anorexia, mucosae pink, bounding pulse.
3	Alsatian	9 mth	F	72 hr	40,2 °C	listless, anorexia, mucosae pink, bounding pulse, splenomegaly.
4	Old English Sheepdog	6 mth	M	12 hr	40 °C	listless, anorexia, mucosae pink, bounding pulse.
5	Labrador	14 a	Fsp	12 hr	40,5 °C	listless, anorexia, mucosae pink, bounding pulse.
6	Labrador	3 a	M	2 w	39,2 °C	capricious appetite, weight loss, pale pink mucosae, splenomegaly, bounding pulse.

mth – month, a – year, M – male, F – female, Fsp – female spayed, hr – hours, w – weeks.

Table 2: SIGNIFICANT CLINICAL FEATURES OF SEVERE CATEGORY

Case No.	Breed	Age	Sex	Duration of symptoms	Rectal temperature	Clinical Findings
7	Dachshund	8 a	M	15 hr	39 °C	Coma, horizontal nystagmus, chemosis, bulbar conjunctival suffusion, dyspnoea, mucosae congested, vomition, 10 % dehydration, rapid weak pulse
8	Nondescript Terrier	12 mth	M	12 hr	40,7 °C	stupor, petechiae gums, dyspnoea, mucosae pink, 5 % dehydration, rapid weak pulse.
9	Alsatian	5 mth	M	48 hr	40,2 °C	depressed, angioneurotic oedema of supraorbital fossae, body and legs, petechiae penis and sclera, bounding pulse, splenomegaly.
10	St. Bernard	4 mth	M	12 hr	40 °C	stupor, dyspnoea, mucosae white, rapid weak pulse, splenomegaly.
11	Bouvier	6 mth	F	24 hr	40,2 °C	stupor, catalepsy, chemosis, anisocoria, vomition, diarrhoea, 5 % dehydration, rapid weak pulse, splenomegaly, pain on abdominal palpation.
12	Pointer	12 a	M	72 hr	40 °C	stupor, hypersalivation, dyspnoea, congested mucosae, vomition, diarrhoea, bounding pulse, splenomegaly.

mth – month, a – year, M – male, F – female, hr – hours.

The most significant difference between the mild and severe category was the high concentration of haemoglobin found to be present in the latter category. Only Case 3 of the four urine specimens examined from the dogs with mild disease had a slight (1+) presence of haemoglobin. All the dogs with severe disease had urine strongly positive (4+ or 5+) for haemoglobin or had macroscopic haemoglobinuria (Cases 7, 8 and 12). Bilirubin was present in 8 of the 10 urine specimens examined (Cases 3, 6, 7, 8, 9, 10, 11 and 12) and was consistently elevated in the severe group.

Protein was detected in all the specimens examined although it was consistently elevated in the severe group. Glucosuria was present only in Case 7 and urobilinogenuria only in Case 11, both dogs having severe disease. The urine pH varied from pH 5 to pH 8 with the severe cases having a consistently lower pH. The urine specific gravity varied from 1,015 to over 1,035.

Table 3: URINE ANALYSIS

Case No.	Nitrite	pH	Protein	Glucose	Ketones	Urobilinogen	Bilirubin	Blood or Haemoglobin	S.G.	Colour	Microscopic examination
1	–	6	1+	–	–	–	–	–	1,030	Yellow	Granular casts, renal tubular epithelium.
2	Not examined										
3	–	8	2+	–	–	–	1+	1+	>1,035	Yellow	–
4	–	7	1+	–	–	–	–	–	>1,035	Yellow	–
5	Not examined										
6	–	7	3+	–	–	–	3+	–	>1,035	Yellow	Granular casts, renal tubular epithelium.
7	–	6	3+	1+	–	–	2+	5+	1,020	Dark red	Granular casts, Haemoglobin casts, erythrocytes.
8	–	5	3+	–	–	–	3+	5+	1,020	Dark red	Granular casts, Haemoglobin casts.
9	–	6	3+	–	–	–	3+	5+	1,025	Dark yellow	Granular casts, Haemoglobin casts, bilirubin pigment.
10	–	5	2+	–	–	–	3+	5+	1,018	Dark yellow	Granular casts, Haemoglobin casts, renal tubular epithelium.
11	–	6	3+	–	–	1+	3+	4+	1,023	Yellow	Granular casts, Haemoglobin casts.
12	–	6	3+	–	–	–	3+	5+	1,015	Dark red	Granular casts, Haemoglobin casts, renal tubular epithelium.

Haematological and Coagulation Findings

The haematological and coagulation findings of the patients at presentation and at subsequent sampling are summarized in Table 4.

All patients exhibited a marked thrombocytopenia at presentation, the average platelet count (Plat) being 20,7 x 10^9 platelets/ℓ of blood (range 4,4 to 33). The mild category had an average platelet count of 25,5 x $10^9/\ell$ of blood (range 16 to 33) while the severe category had an average count of 15,9 x $10^9/\ell$ of blood (range 4,4 to 30).

The prothrombin time (PT) was significantly increased only in Case 10. According to Kociba[24] the PT should be considered abnormally prolonged when 5 or more seconds greater than a normal canine control.

The activated partial thromboplastin time (APTT) was prolonged in 7 of the 12 dogs. Two dogs with mild disease (Cases 1 and 4) and 5 of the 6 dogs (Cases 7, 8, 10, 11 and 12) with severe disease had prolonged APTT. According to Kociba[24] the APTT should be considered abnormally prolonged when 7 or more seconds greater than a normal canine control. In the present series the APTT was delayed by an average of 15,4 seconds (range 11 to 20) when compared with the average for normal canine controls[34].

Fibrinogen levels (Fib) were determined in 11 of the 12 dogs. Only in Cases 3, 6 and 8 were the levels in the normal range of 1 to 5 gm/ℓ[34 44]. In Cases 1, 2, 4, 5, 7, 9, 11 and 12 elevated levels were present with an average of 5,995 gm/ℓ (range 5,28 to 7,31 gm/ℓ).

Fibrinogen degradation products (FDP) were determined in each of the 12 dogs investigated. All 6 dogs with mild disease had values of less than 10 mg/ℓ blood. Five of the 6 dogs with severe disease had FDP values greater than 10 mg/ℓ of blood, while Case 12 had a value of less than 10 mg/ℓ of blood. Three dogs (Cases 8, 10 and 11) had values of FDP greater than 40 mg/ℓ of blood, Case 9 had a value of 25 mg/ℓ while Case 7 had a value of 20 mg/ℓ of blood at initial examination. The normal value for FDP in dogs is less than 10 mg/ℓ of blood[16].

Total leukocyte (WCC) and differential counts were established for each dog in the series. The total and differential counts varied widely. At initial examination a normal leukocyte count was present in Cases 3, 6, 8, 9, 10, 11 and 12, while a leukocytosis (absolute neutrophilia) was present in Case 1 and a leukopenia was present in Cases 2, 4, 5 and 7. A relative neutrophilia was present in Case 5. The mean total leukocyte count for the mild category was 10,65 x $10^9/\ell$ of blood (range 4,9 to 18,8 and for the severe category it was 10,1 x $10^9/\ell$ of blood (range 3,8 to 15).

The erythrocyte count (RCC) and haemoglobin concentration (Hb) were evaluated in 11 of the 12 dogs, while the packed cell volume (PCV) was determined in all cases. The mean erythrocyte count for the mild category was 4,48 (range 2,4 to 6,5) x $10^9/\ell$ of blood and for the severe category 4,5 (range 1 to 7,6) x $10^9/\ell$ of blood. The mean haemoglobin concentration for the mild category was 114,7 (range 74 to 167) gm/ℓ and that for the severe category 104,8 (range 27 to 187) gm/ℓ. The mean packed cell volume for the mild group was 0,325 (range 0,2 to 0,48) while that for the severe group was 0,358 (range 0,09 to 0,63). The discrepancy in PCV value of the mild versus the severe category when compared to the erythrocyte count, is due to the inclusion of Case 12 (which had an abnormally high PCV) into the mean PCV of the severe group. Erythrocyte count and haemoglobin determinations were not performed on Case 12. When the PCV of Case 12 is excluded, the mean PCV of the severe group is 0,304 (range 0,09 to 0,52).

Table 4: HAEMATOLOGICAL AND COAGULATION FINDINGS

Case No.	1[e]		2[e]		3[d]	4[d]		5[d]		6[e]		7[e]	8[e]		9[e]		10[e]			11[d]			12[d]		Normal Range
Day	1	2	1	2	1	1	2	1	2	1	3	1	1	5	1	3	1	2	4	1	3	6	1	2	
PT patient	6,4	6	8	6	8	9		8,4		7,25		7	7		6		12	10	9,9	9,6	9,5	10,3	9,2		
Control	6	6	6	6	7,4	6,8		8		6		7	7		6		6	6	9,2	9	9	9	8,5		6 – 14 sec.[a]
APTT patient	45	32	31	33	21,6	27,5		20		27		38	41	38	28		40	47	29	25,2	24,2	28	34,5		
Control	25	25	33	33	15,1	15,4		16,4		25		25	22	25	25		25	22	24	14,2	14,2	15	16,8		15 – 25 sec.[b]
Fib	5,28		5,45	4,8	1,23	5,44	-	7,31	-	2,03		5,28	4,35	9,5	6,3					6,7	5,4	4,62	6,2		1 – 5 gm/ℓ[a]
FDP	<8		<8	<8	<10	<10		<10		<8		20	>40	10	25	<8	>40	40	<8	>40	<10		<10		<10 mg/ℓ[c]
Plat.	25		16	110	23	31	35	25	80	33	57	10	14	27	23	71	4,4	71	110	14	17	25	30	92	200 – 500 x 10^9/ℓ[a]
WCC	18,8		5,2	12	15	5,7	15,3	4,9	12	14,3	7,5	3,8	10,7	22,5	6	24	15,1	28,6	30,5	11,6	15	13	13,4	22,8	6 – 17 x 10^9/ℓ[a]
RCC	3,11		6,5	5,8	3,95	4,55	4	6,4		2,4	3,6	7,6	3,4	6,4	4,5	4,9	1	4,3	4,1	6	4	4,6			5,5 – 8,5 x 10^{12}/ℓ[a]
Hb	74		168	146	101	110	102	161		74	90	187	82	164	108	130	27	105	99	120	78	83		187	120 – 180 gm/ℓ[a]
PCV	0,23	0,26	0,48	0,43	0,28	0,31	0,3	0,45	0,44	0,2	0,29	0,52	0,27	0,46	0,31	0,36	0,09	0,32	0,32	0,33	0,23	0,27	0,63	0,52	0,37 – 0,55[a]
Neut	0,89		0,6	0,69	0,61	0,74	0,64	0,9	0,74	0,64	0,71	0,62	0,67	0,88	0,68	0,79	0,76	0,81	0,78	0,8	0,8		0,82	0,8	0,67 – 0,77[a]
Mono	0,03		0,03	0,07	0,11	0,04	0,06	0,02	0,05	0,13	0,13	0,05	0,03	0,02	0,04	0,05	0,08	0,06	0,03	0,05	0,12		0,07	0,09	0,03 – 0,1[a]
Lympho	0,08		0,37	0,24	0,28	0,22	0,3	0,08	0,21	0,2	0,16	0,33	0,3	0,1	0,28	0,16	0,16	0,13	0,19	0,15	0,08		0,1	0,1	0,12 – 0,3[a]
Eosino										0,03		0,01											0,01	0,01	0,02 – 0,1[a]

[a] Normal ranges from Schalm O W, Jain N C, Carrol E J 1975

[b] Normal range from Moore D J 1978

[c] Normal range from Greene C E 1975

[d] Values determined by the Department of Haematology South African Institute for Medical Research, Johannesburg

[e] Values determined by Clinical Laboratories, Jeppe Street, Johannesburg

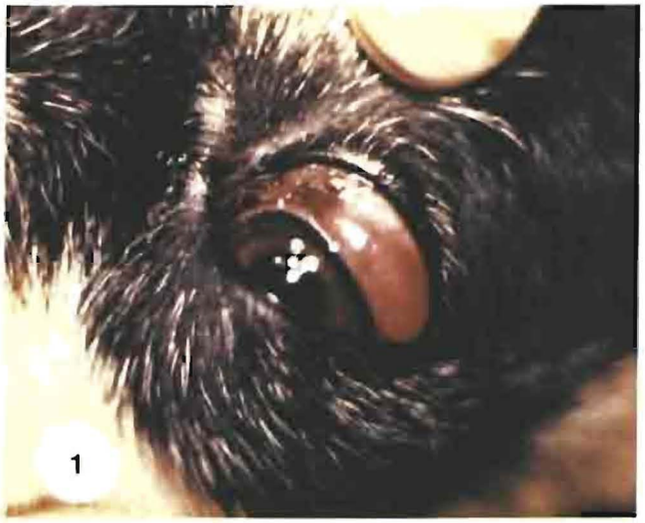

Fig. 1. Chemosis and conjunctival haemorrhage.

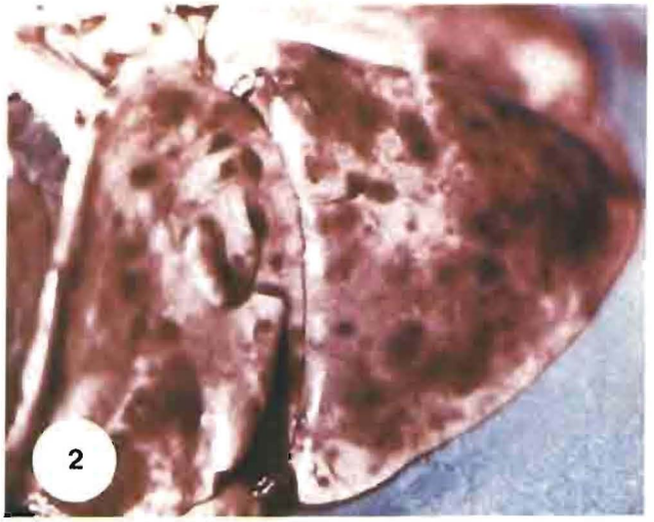

Fig. 2. Pulmonary oedema, emphysema and haemorrhage.

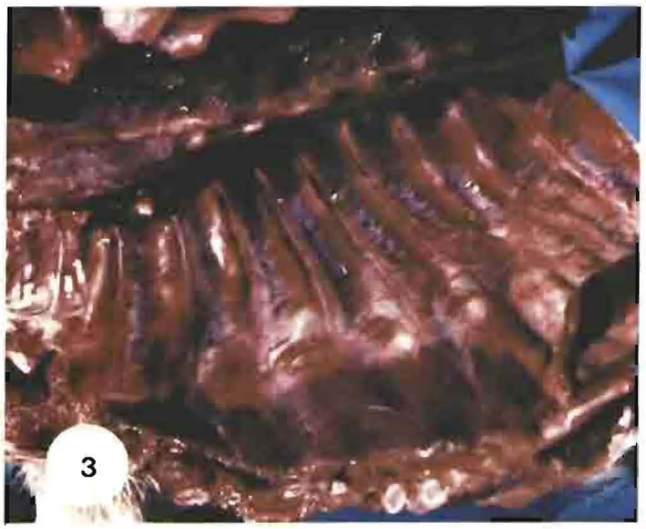

Fig. 3. Haemorrhage of the parietal pleura and intercostal muscles.

Fig. 4. Haemorrhage of the diaphragm.

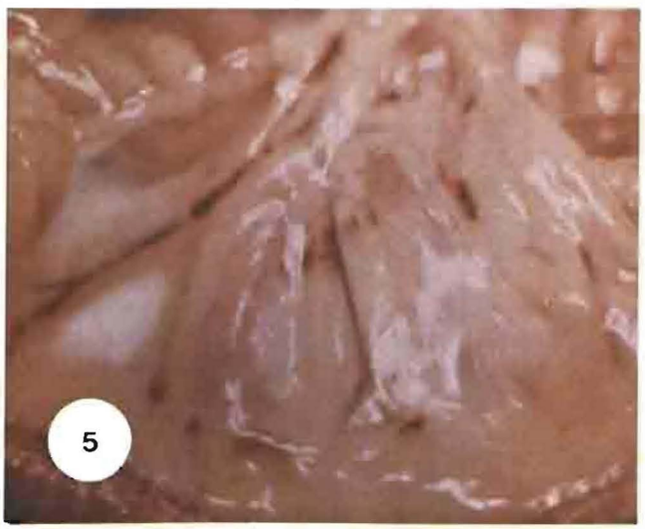

Fig. 5. Haemorrhage of the mesentry.

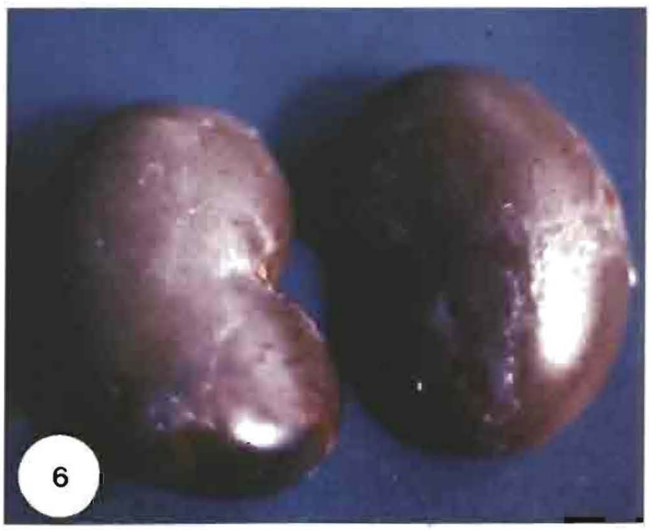

Fig. 6. Renal cortical petechiae and a posterior polar infarct of the left kidney.

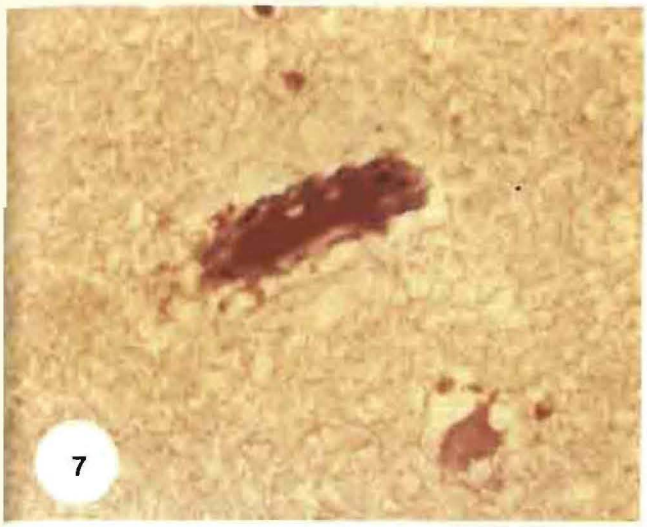

Fig. 7. Photomicrograph of a section of the cerebral white matter illustrating an arteriolar fibrin thrombus and periarteriolar rarefaction. (MSB stain, 500 x).

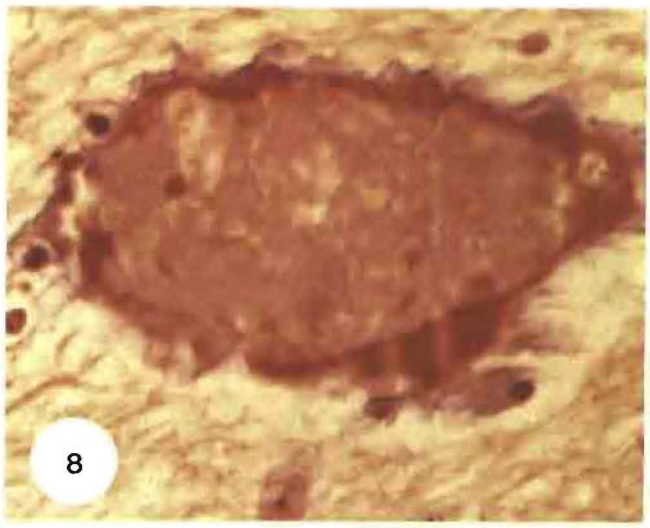

Fig. 8. Photomicrograph of a section of the cerebellar white matter illustrating a small vein with central stasis and peripheral fibrin deposition. (MSB stain 500 x).

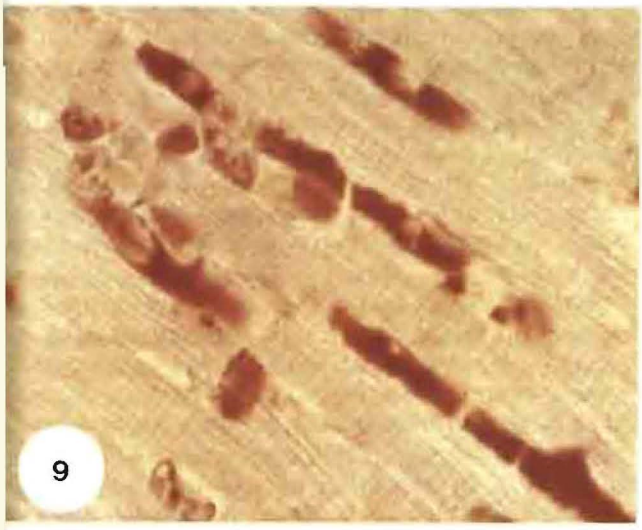

Fig. 9. Photomicrograph of a section of the myocardium illustrating capillary fibrin thrombi. (MSB stain, 2000 x).

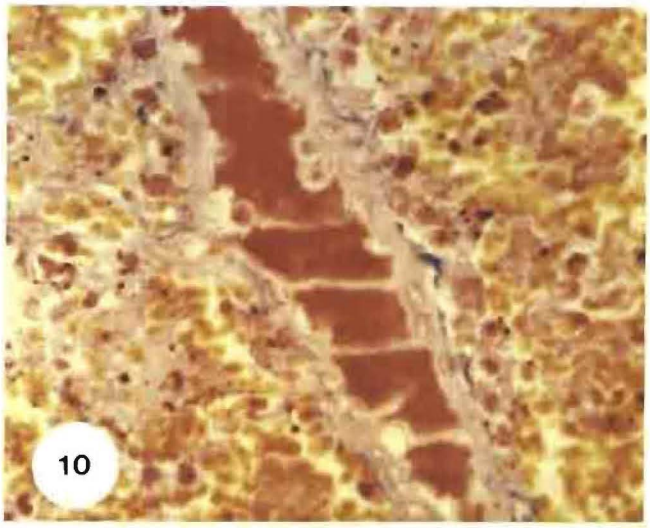

Fig. 10. Photomicrograph of a section of lung illustrating an arteriolar fibrin thrombus and haemorrhage (MSB stain, 500 x).

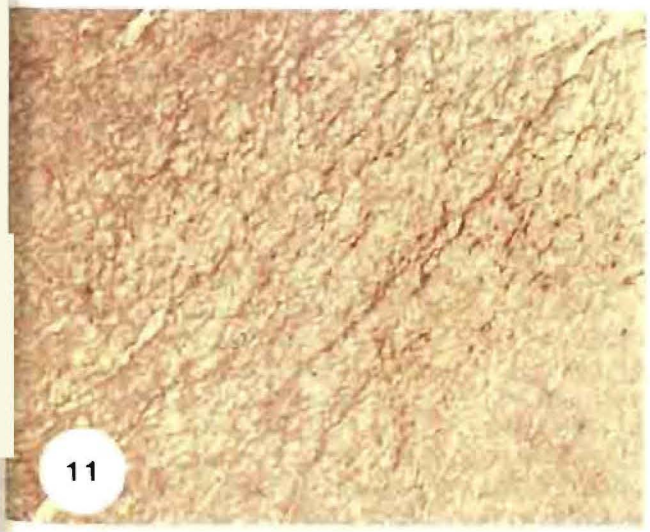

Fig. 11. Photomicrograph of a focal area of intestinal smooth muscle illustrating cloudy swelling, hydropic degeneration and capillary fibrin thrombi. (MSB stain, 500 x).

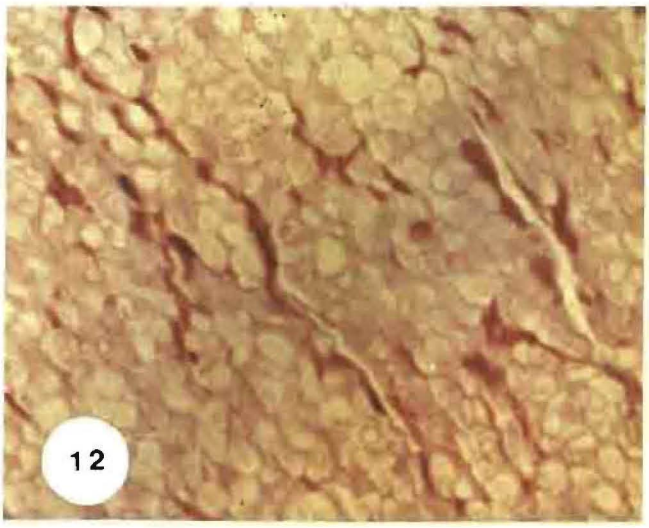

Fig. 12. Photomicrograph of a focal area of intestinal smooth muscle illustrating cloudy swelling, hydropic degeneration and capillary fibrin thrombi. (MSB stain, 1250 x).

Haemoglobinaemia was absent in the dogs of the mild category but present in 5 of the 6 dogs (Cases 7, 8, 10, 11 and 12) in the severe category.

Autopsy Findings

Post mortem examinations were performed on Cases 7 and 8 within 2 hours of death. Case 7 died 4 hours after presentation while Case 8 died 5 days after presentation.

Macroscopic Pathology of Case 7.

On external examination of the carcass the only significant change observed was a marked bilateral chemosis, petechiae and suffusion of the bulbar conjunctivae (Fig 1). General congestion and cyanosis without any apparent signs of anaemia or icterus were noticed. At autopsy the following were observed in the thoracic cavity: a severe degree of pulmonary oedema, emphysema and haemorrhage (Fig. 2); a slight hydropericardium; subepicardial and subendocardial petechiae and ecchymoses; severe suffusion of the parietal pleura and intercostal muscles (Fig. 3); severe ecchymoses and suffusion of the mediastinum; petechiae and ecchymoses of the diaphragm (Fig. 4). In the abdominal cavity the following were observed: hepatomegaly; splenomegaly; suffusion of the subserosal surface of the stomach; oedema, petechiae and ecchymoses of the pancreas; bilateral renal cortical petechiae. The brain was severely congested with a focal haemorrhagic area of the right occipital lobe and cerebellum.

Macroscopic Pathology of Case 8.

No significant changes were observed on external examination of the carcass and no signs of anaemia or icterus were present. At autopsy a generalized congestion was present with severe congestion of the brain. The most significant thoracic findings were: severe pulmonary oedema, emphysema and haemorrhage; severe suffusion of the parietal pleura and intercostal muscles. The abdominal observations were: petechiae and ecchymoses of the duodenum and ileum; an ulcer 5 mm in diameter in the duodenum; petechiae and ecchymoses of the mesentery (Fig. 5); oedema, petechiae and ecchymoses of the pancreas; bilateral renal cortical petechiae; infarction of the posterior left renal cortex (Fig. 6).

Microscopic pathology

Histopathological examination of various organs and tissues from both cases revealed the presence of fibrin microthrombi with Martius-Scarlet-Blue stain but were not detected in Haematoxylin-Eosin stained sections.

In Case 7 fibrin microthrombi together with congestion, oedema and haemorrhage were detected in the cerebrum (Fig. 7), cerebellum (Fig. 8), myocardium (Fig. 9), lung (Fig. 10), diaphragm, spleen, liver, kidney cortex and medulla, ciliary body and rete testis.

In Case 8 fibrin microthrombi of the arterioles and capillaries together with congestion, oedema and haemorrhage were detected in the cerebrum, cerebellum, lungs, myocardium, kidney and small intestine (Figs. 11 and 12).

DISCUSSION

Stevenel's Blue Stain was found to be a reliable and fast stain for the identification of *B. canis*. It stained erythrocytes light blue, nuclei purple and *B. canis* internal structure light purple with a dark purple border[34].

The mean duration of symptoms in the mild group (82 hours) when compared to the severe group (30,5 hours) would suggest that, in general, severe cases of canine babesiosis are more acute than mild cases[34].

Criteria are presented which categorize dogs with *B. canis* infection into mild (uncomplicated) or severe (complicated) disease on a clinical basis and these criteria are substantiated with coagulation profiles[34].

Symptoms observed only in the severe category include haemorrhages on the mucous membranes, angioneurotic oedema, nervous symptoms (stupor, coma, depressed or absent spinal and postural reflexes, catalepsy, anisocoria and nystagmus), rapid weak pulse, dehydration, haemoconcentration, dyspnoea, vomition and diarrhoea. These symptoms would therefore, when exhibited alone or in combination be sufficient criteria to categorize the dog as having severe babesiosis[34].

The urine examination reveals that significant renal injury occurs in both mild and severe *B. canis* infection, and this finding is in agreement with results previously published[25 28]. The most striking difference between the two groups is the consistent presence of increased quantities of haemoglobin in the urine of severely affected dogs. The presence of significant (4+ and 5+) haemoglobinuria or macroscopic haemoglobinuria should thus categorize dogs with biliary fever as having severe disease[34].

A thrombocytopenia was evident in both categories of dogs although it was more marked in the severe group[34]. Wilkens *et al.*[49] indicate that patients with platelet counts of less than $50 \times 10^9/\ell$ of blood are liable to spontaneous haemorrhage and thus a thrombocytopenia of the degree found in the severe category of dogs would alone be responsible for initiating haemorrhage. In a thrombocytopenic state, as the patelet count decreases, blood vessel integrity decreases proportionately and the possibility of escape of erythrocytes through the capillary wall increases[44]. Pressure stresses on blood vessel walls increase the likelihood of haemorrhage[44] and it is indeed fortunate that most dogs with biliary fever are lethargic which thus reduces traumatic injury to blood vessels.

Thrombocytopenia in protozoal infections in man has been described in *Trypanosoma rhodesiense* infection[42] and *Plasmodium falciparum* malaria[46]. The mechanisms of thrombocytopenia in these infections may involve splenic pooling of platelets, removal of normal platelets by an abnormally avid reticulo-endothelial (RE) system, the removal of immunologically damaged platelets by a normal RE system, DIC or a combination of the above[42 46]. Thrombocytopenia is a consistent finding of DIC in both man and dog[1 14 16 32 47]. Thrombocytopenia in *B. canis* infection has not previously been reported and although the mechanism remains unresolved, platelet kinetic studies may distinguish which of the above phenomena are active although DIC is assumed to aggrevate the thrombocytopenic state in dogs with severe *B. canis* infection[34].

Malherbe[28] used the prothrombin time (PT) as an estimation of liver function in canine babesiosis and although the test was frequently prolonged, it was

noted that the elevated levels returned to normal despite obvious hepatocellular damage. Malherbe[28] concluded that the hepatocellular injury was the only likely reason for the elevated PT values and that the haemorrhagic diathesis frequently observed in canine babesiosis was due to the deficiency in "prothrombin". Evaluation of DIC in patients with severe liver disease may be particularly difficult because of the frequent association of excessive fibrinolysis and/or depletion of clotting factors synthesized in the liver[1 7]. Liver and splenic dysfunction may place a patient in a special category of risk to developing DIC[7]. Because the PT has been found to be infrequently raised in the present series of dogs, the extrinsic clotting system, of which the PT is an indicator, is thought to be stimulated after the intrinsic clotting system although the time of blood collection may be critical in establishing an abnormal value. The present study would indicate that a prolonged PT in biliary fever may indicate the presence of DIC[34].

The activated partial thromboplastin time (APTT) has not previously been determined in *B. canis* infection[34]. The finding of prolonged APTT particularly in severe cases is interpreted as an indication of DIC[34]. The intrinsic clotting system, of which the APTT is an indicator, would appear to be more susceptible to stimulation in biliary fever than the extrinsic system[34]. This is possibly due to the hypoxic effect created in the microvasculature as a result of erythrocyte sludging[23]. This hypoxic injury would damage the vascular endothelium and stimulate the intrinsic clotting system[12 47]. The prolonged APTT determined in two dogs with mild disease (Cases 1 and 4) indicates that a deranged coagulation mechanism is present in some dogs with uncomplicated disease[34]. However, as the categorization of patients was not absolute, the two cases which appeared to have mild disease may have been in transition to severe disease[34].

Fibrinogen depletion is a finding of DIC in man[32 47] and dog[18 24] but is a less sensitive indicator of DIC in the dog[16 30]. Depletion of the coagulation factors may be transient and is often followed by supranormal levels[1 4 36]. The finding of supranormal levels in the present series confirms the lack of specificity of fibrinogen levels in DIC induced by *B. canis* infection in the dog[34].

Increased levels of fibrinogen degradation products (FDP) is regarded as an accurate method of determining the late phenomenon of excessive fibrinolytic activity in DIC[7 16 47]. Increased levels are usually found in patients with complicated *P. falciparum* malaria, particularly patients with cerebral malaria[22 41]. However, some patients with cerebral malaria and DIC may have normal levels of FDP[4]. Levels of FDP greater than 40 mg/ℓ of blood are probably unequivocal evidence of DIC in biliary fever[34]. Levels between 10 and 40 mg/ℓ are questionable evidence of DIC, although Case 7 with a level of 20 mg/ℓ subsequently died and at autopsy was confirmed as having DIC[34].

The leukocyte count in canine babesiosis is variable[6 20 35] although in severe cases a leukocytosis with absolute neutrophilia is frequently present[28]. In DIC the leukocyte count varies considerably[32 47] although a leukopenia is frequently observed[18 30 50]. In the present series 4 dogs exhibited a leukopenia, one of which died (Case 7) and at autopsy was confirmed as having DIC. Although the leukocyte and differential counts in the present series are inconclusive, a leukopenia in *B. canis* infection should be viewed as a grave prognostic sign, as the physiologic response to a stressful situation in the dog should be a leukocytosis exhibited as a neutrophilia and monocytosis[34].

Anaemia is a commonly reported phenomenon in biliary fever[25 28 35 48] and is considered progressive and responsive[25]. Anaemia is frequently a consequence of DIC[32 47] as is hypovolaemic shock[32]. Both the mild and severe categories in the present series incorporated dogs which were severely anaemic and it is thus irrational to arbitrarily categorize dogs with biliary fever according to the degree of anaemia as envisaged by Malherbe[28]. It is proposed that the categorization, if at all possible, should evaluate the patient in its entirety and not merely piecemeal. The unreported phenomenon of haemoconcentration (Case 12) in *B. canis* infection is suggested as a grave prognostic sign as it may indicate hypovolaemic shock[34].

DIC causes microangiopathic haemolysis as a result of physical injury and fragmentation of the erythrocytes due to the presence of deposited fibrin which obstructs the microvasculature[32 47]. In *B. canis* infection, mechanical destruction of infected erythrocytes by the parasites together with immunologic factors results in intravascular haemolysis[25]. It is proposed that the extent of one or more of the above mechanisms is of such a magnitude in severe biliary fever that macroscopic haemoglobinaemia becomes evident[34].

DIC is characterized microscopically by the presence of widespread microthrombi, particularly in the arterioles and capillaries[32 45 47]. Fibrin microthrombi are not readily appreciated in routine Haematoxylin-Eosin stained sections and specific fibrin stains must be used to detect their presence[45]. A major problem in the histopathologic diagnosis of DIC is the rapid ante- and postmortem dissolution of the thrombi[16 32 45 47]. In situ there is a rapid activation of fibrinolysis secondary to coagulation which occurs at a similar rate, whether or not the animal survives the episode of DIC[16 47].

Fibrin microthrombi were detected in various organs of both autopsies (Cases 7 and 8) using Martius-Scarlet-Blue stain but were not detected in sections stained with Haematoxylin and Eosin[34]. Although Case 8 responded initially to therapy as illustrated by the haematological and coagulation profiles returning toward the normal range (Table 4), the dog died acutely 5 days after presentation. At autopsy the anatomico-pathological features of DIC were confirmed microscopically despite the initial clinico-pathologic improvement. The detection of fibrin microthrombi serves to prove the presence of DIC observed clinically. DIC thus plays a significant role in the pathogenesis of the severe disease in *B. canis* infection[34].

Of the previously reported "atypical" symptoms of biliary fever[2 5 26 27 38 39] nervous, respiratory, circulatory and gastrointestinal manifestations were observed in patients with severe disease in the present study. It is suggested that these symptoms may have been a manifestation of DIC localized primarily to a particular organ system[34].

The mechanisms whereby *B. canis* induces DIC are presently unknown, although one or more of the following factors could be involved:-

1. Hypoxic endothelial injury caused by sludging of blood cells.
2. Intravascular haemolysis which releases erythrocyte thromboplastin.

3. Platelet lysis which releases platelet thromboplastin.
4. Tissue injury which releases tissue thromboplastin.
5. Blockade of the reticuloendothelial system.

It is curious that coagulation and fibrinolysis have rarely been studied in relation to *Babesia canis* infection in the dog and these preliminary findings are published to stimulate interest in the field.

CONCLUSIONS

The naturally infected clinical cases of *Babesia canis* infection presented in this study are categorized into mild (uncomplicated) and severe (complicated) forms. The severe form exhibited many of the important clinical, coagulation and haematological, pathological and histopathological features of DIC described in the literature. On the basis of these findings, there can be little doubt that DIC may be a serious complication of severe *B. canis* infection in the dog. Thrombocytopenia is determined as being present in both mild and severe *B. canis* infections.

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BOOK REVIEW

BOEKRESENSIE

FUNCTIONAL MAMMALIAN NEUROANATOMY

T.W. JENKINS, 2nd Edition
Lea & Febiger, Philadelphia. 1978. pp. XXII + 480. Figs 165.
Price not stated (approx. R25).

Although the average practitioner may have lost interest in the nervous system owing to his difficulty in understanding it while a student, or owing to lack of proper teaching of this subject during his veterinary course, this book could help him regain confidence in its study. For those more especially interested in neurological problems it will prove a most useful background text. The veterinary student, perhaps more than anyone else, will find it extremely valuable, but it would be a *handful* for him. The veterinary profession has had to rely mainly on texts based on the human when they wish to study neuroanatomy. This work, the second of its kind on veterinary neuro-anatomy, makes an excellent text available to the veterinarian. The emphasis is on functional anatomy, the text is very easy to read, and is exceptionally well illustrated. The author has managed the anatomical aspects admirably. There is enough topography, including an atlas at the end of the book, to enable one to find one's way around the brain without being smothered in minutiae. Abnormal function is particularly well reviewed, with useful comments on the effects of natural and experimental lesions. In the second edition the author has adopted the nomenclature based on *Nomina Anatomica Veterinaria* – a great improvement on the first edition.

The text deals briefly with the evolution, embryology and micro-anatomy of the nervous system before describing the vascular and meningeal arrangements. Anatomy and physiology of the spinal cord are followed by regional descriptions of the brain. Chapters on the cranial nerves, the eye and visual system and on the ear lead into the third part which is a photographic atlas of sections of the central nervous system of the dog. All in all, the book is a valuable addition to the veterinary literature.

J.M.W. le Roux

SAVV GOUE MEDALJE: AGSTE TOEKENNING 1979
LOURENS WEPENER VAN DEN HEEVER

Die Goue Medalje van die SAVV word vanjaar toegeken aan Prof L.W. van den Heever ter erkenning van sy wetenskaplike prestasies en sy bydraes tot vooruitgang van die veeartsenykundige wetenskap.

Lourens Wepener van den Heever is op 18 Augustus 1923 in Pretoria gebore en matrikuleer in 1939 aan die Afrikaanse Hoër Seunskool in dieselfde stad. Hy het reeds as voorgraadse student in die veeartsenykunde te Onderstepoort uitsonderlik presteer deur sy BVSc-graad in 1944 met lof te verwerf. Die gesogte Theiler gedenkmedalje van die S.A. Biologiese Vereniging is dan ook vir sy akademiese prestasies aan hom toegeken. Later in sy loopbaan sit hy sy studies voort en behaal die D.V.V.G. in 1963 en die M. Med. Vet. (Hyg)-graad in 1970, alles aan die Universiteit van Pretoria.

Nadat hy as veearts gekwalifiseer het volg hy 'n loopbaan wat vinnig in die rigting waarin hy hom tans bevind, naamlik veterinêre volksgesondheid, beweeg en wat deurspek is van prestasies.

Prof van den Heever het die wetenskap van veterinêre volksgesondheid in S.A. bevorder soos niemand voor hom dit kon vermag het. As die eerste voltydse stadsveearts van die Gesondheidsdepartement in Germiston waar hy vanaf 1946 vir bykans 13 jaar werksaam was het hy besondere goeie diens gelewer. Hy verwerf dan ook 'n "Fellowship" van die Wêreld Gesondheid Organisasie in 1959 wat hom in staat stel om voedselhigiëne in Engeland, Denemarke, Duitsland, Holland, Italië en Kenia te bestudeer.

Die kennis en ondervinding wat hy so opgedoen het, het hy op velerlei gebiede wat op sy vak van toepassing is tot diens van die wetenskap gestel.

Hy het navorsing op 'n gevorderde vlak op 'n wye verskeidenheid aspekte van vleis- en melkhigiëne gedoen en die resultate in 56 artikels (49 wetenskaplik en 7 populêr-wetenskaplik) gepubliseer.

Van sy bekendste navorsing handel oor die diagnose en ander aspekte van mastitis, en hy het heelwat voorligting in verband met die beheer van die siekte op 'n kudde-basis gedoen. Hy het pionierswerk op die effek van spoorvervoer op slagvee gedoen. Gesondheidsaspekte van biltong het heelwat van sy aandag geniet. Dit het onder andere die lewensvatbaarheid van masels, *Salmonella* bakterieë en verskeie mikro-organismes in biltong ingesluit. Hy was verantwoordelik vir die ontdekking dat *Parafilaria bovicola* in S.A. voorkom. Hy het pionierswerk op die preservering van afval as eetbare produk gedoen. Hy het die situasie in S.A. wat betref die teenwoordigheid van *E. coli* stamme met oordraagbare weerstand teen antibiotika nagegaan. En so kan mens aangaan om oor die navorsingsaktiwiteite van hierdie veelsydige wetenskaplike verslag te doen.

Prof van den Heever het vir etlike jare die navorsing van die Navorsingsinstituut vir Veeartsenykunde, Onderstepoort in melk- en vleishigiëne gelei en 'n afdeling tot stand gebring wat nasionale en internasionale bekendheid verwerf het.

Hy is sedert 1960 leerkrag in Voedselhigiëne en Volksgesondheid aan die Fakulteit Veeartsenykunde aan die Universiteit van Pretoria. Hy was verantwoordelik vir die instelling van die D.V.V.G., wat benewens die D.V.Sc die eerste nagraadse kursus was. Vanaf 1973 is hy die eerste voltydse professor en hoof van die afdeling. In dié tyd het hy 'n groot aantal voorgraadse asook nagraadse studente in sy vakgebied opgelei. Hy het dus 'n onuitwisbare invloed op dié vakgebied soos dit in S.A. beoefen word gehad. Sy besondere belangstelling in die soönoses het hierdie begrip 'n ope boek by sy studente en kollegas gemaak.

Hy het op nie minder as 6 kommissies en komitees van ondersoek oor verskillende aspekte van vleis- of voedselhigiëne gedien nie en dien steeds op sommige van hulle. Hy was bv. vanaf 1972–1977 lid van die Abattoir-kommissie en vanaf 1972–1973 voorsitter van die komitee wat ondersoek ingestel het na die toepassing van die *Wet op Higiëne by Diereslag, Vleis en Vleisprodukte* (Wet nr 87 van 1967). Hierdeur het hy die saak van veterinêre volksgesondheid op onbaatsugtige wyse gedien en kon hy veel daartoe bydra om die vete-

rinêre wetenskap se rol daarin te bevorder en te verskans. Sedert 1963 is hy lid van die SABS se komitee vir Ingemaakte en Oop Verpakte Vleisprodukte. Hy dien voorts sedert 1977 in die Raad op Atoomkrag se komitee vir Stralingstoepassing in die Landbou.

Hy dien sedert 1953 op die redaksie-komitee en was vanaf 1975 tot 1978 voorsitter van die redaksiekomitee van die joernaal van die SAVV, 'n besonder veeleisende taak waardeur hy 'n ontsaglike bydrae gemaak het tot die propagering en bekendstelling van die veeartsenykunde as beroep en as wetenskap.

Deur sy lidmaatskap van wetenskaplike verenigings buite die SAVV soos die S.A. Vereniging van Suiweltegnologie waarvan hy die President is; deur sy betrokkenheid by komitees, takke en groepe binne die SAVV soos die Uitvoerende Komitee, die Raad van die SAVV, en die Veeartsraad; deur sy diens as Onderpresident en President van die SAVV vanaf 1966 tot 1972, as organiseerder van kongresse en simposia, dien hy nie slegs sy professie nie, maar ook die veterinêre wetenskap feitlik daagliks op 'n besonder onbaatsugtige wyse.

Benewens die toekennings waarna reeds verys is, ontvang hy ook 'n "Fellowship" van die Royal Society of Health in 1959 en die Boswell-Toekenning van die SAVV in 1977.

Dwarsdeur sy loopbaan as veearts en spesialis in veterinêre volksgesondheid het prof van den Heever op sy kenmerkend beskeie maar besonder doelgerigte, vasberade wyse die saak van die veeartsenykundige wetenskap bevorder, net soos hy ook die veterinêre professie gedien het. As die eerste lid van die "jonger geslag" van veeartse wat hierdie groot eer te beurt val is hy 'n besonder verdienstelike wenner van die gesogte Goue Medalje van die SAVV.

BOOK REVIEW

BOEKRESENSIE

VIRAL AND BACTERIAL ZOONOSES

C H ANDREWS AND J R WALTON
ISB 0 7020 0632 7
Bailliere Tindal, London, pp. XIV + 161, Fig 7, Tabs 2.

Like its partner "The Control of Disease", this paper-back volume is one of a series of four on *Animals and Human Health* under the general editorship of G.C. Brander. The aim of the volume is to consider various key aspects of the interrelationship of diseases affecting man and animals and by promoting a better understanding to also suggest how the health and well being of man and animals may be enhanced.

This book surveys a wide field in a limited number of pages and consequently does not treat the matter in great depth. Readers are referred to specifically detailed, further reading matter for fuller information. Apart from dealing briefly with specific infectious agents, the book furnishes excellent general philosophy and background under the headings "Introduction", "The Natural History of Zoonoses" and "Transmission of Infection".

The book will be useful to both medical and veterinary students and practitioners as well as persons working in public health, food manufacture, the pet and livestock industries and agriculture in general.

L.W. v.d. H.

THE BOSWELL AWARD FOR 1979

BASIL HENRY PAPPIN

The winner of The Boswell Award for 1979 is Basil Henry Pappin. The Award is given in recognition of his excellent and dedicated services to the veterinary profession, to his calling as veterinarian and to the SAVA. The dedication with which Dr Pappin has served his profession and his calling is indeed so well known to every veterinarian in SA that it hardly requires a motivation. The high-lights of his career in terms of his eligibility for the Award are outlined in this citation.

Basil Pappin was born in Johannesburg on 12th November 1924 and matriculated at Pretoria Boys High School. He qualified at the Veterinary Faculty, Onderstepoort in 1946 and was awarded the much sought after Theiler and Clinical Medals. He then set out into private practice to become one of the pioneers in this field of veterinary enterprise in South Africa.

After serving as an assistant in private practice in Johannesburg from 1947 to 1949, he established his own practice in Germiston in 1949. This has been developed into the well known Germiston Veterinary Hospital, which boasts 6 partners, largely through his dedication and enterprise. His staunchest supporter in these ventures has been his wife Agnes whom he married in 1953. The couple have raised a charming family of 2 sons and 3 daughters.

Basil has always endeavoured to provide professional services of the highest standard. To achieve this objective he has propagated continued veterinary education very strongly at every possible level, including practice, branch, group and association levels, and at every opportunity. In this way he has made a tremendous personal contribution to the generally acknowledged sophisticated standard that small animal practice has reached in South Africa.

He has served his profession selflessly by his participation in the widest possible spectrum of Association and other veterinary bodies, both locally and overseas. He was President of the SAVA from 1974–1976 and has been a Council member from 1970 to the present. He is Chairman of the Veterinary Foundation and the National Animal Welfare Committee. He serves on the Executive Committee, Advisory Committee on Veterinary Ethics, Educational Committee, Awards Committee and Veterinary Act Committee of the Association. He was founder of the Clinicians' Group of the Witwatersrand Branch of the SAVA in 1965 and Chairman of the Witwatersrand Branch of the SAVA from 1970–1972. He also represents the Clinicians' Group on the Executive of the World Small Animal Veterinary Association.

He is known for the enthusiasm, zeal and dedication which he puts into every association task and for the comprehensiveness and reliability of his records system. This he manages to accomplish despite the demands which a busy practice makes on his time.

He has filled the most exacting Association position namely that of President with great distinction. Not only did he pull more than his weight at all times, but he launched several successful projects. Most important of these, from the profession's point of view, was perhaps the creation of the post of Director of the Association. He was universally liked and consequently a very successful President.

Basil has now taken on an even more difficult task namely that of Chairman of the Board of Trustees of the Veterinary Foundation after a successful campaign to get the Foundation firmly under the wing of the Association. With his pleasant personality and characteristic perseverance he should have no difficulty in getting this fledgling of the Association off to a successful start.

He is also very active outside the veterinary profession, especially in wildlife and charity matters, and is an eminent and respected member of his community.

Basil Pappin is certainly a very eligible recipient of the Boswell award.

TOEKENNING **AWARD**

SENIOR KAPTEIN SCOTT GEDENK MEDALJE VIR 1978: SUID-AFRIKAANSE BIOLOGIESE VERENIGING

PIETER ARNOLDUS BASSON

Pieter Arnoldus Basson is op 20 Mei, 1931 in die distrik Grootfontein van Suidwes-Afrika gebore. Hy voltooi sy primêre skoolopleiding op 'n plaasskooltjie, in dieselfde distrik, en matrikuleer aan die Paarl Boy's High School in 1949. Hy studeer vir sy eerste jaar veeartsenykunde aan die Universiteit Stellenbosch gedurende 1950. Vanaf 1951 tot 1954 is hy student in die Fakulteit Veeartsenykunde van die Universiteit Pretoria te Onderstepoort. Akademies presteer hy deurgaans ver bo die gemiddelde. In sy finale jaar tree hy op as huisvoorsitter in die koshuiskomitee van Onderstepoort en as kaptein van die eerste rugbyspan.

Nadat hy as veearts gekwalifiseer het, is dr Basson terug na Suidwes-Afrika waar hy as jong staatsveearts reeds besondere prestasies lewer en daardeur sy belofte as wetenskaplike toon. Ten spyte van 'n kwaai lading van regulatoriese diens, o.a. as beampte in bevel van 2 bek-en-klouseer-kampanjes, begin hy intensief navorsing doen op tergende lokale probleemsiektes.

Sy intieme kennis van nie net die veeboerdery in Suidwes-Afrika nie, maar ook die inheemse diere- en plantlewe het ongetwyfeld as agtergrond en motivering vir veel van sy navorsingswerk gedien. Gepaard hiermee besit hy 'n uitsonderlike waarnemingsvermoë en 'n buitengewone toewyding en werksvermoë wat met groot vrug aangewend word.

Sy eerste groot wetenskaplike bydrae maak hy op 'n siekte bekend as uitpeuloog deur te bewys dat dit 'n miase is wat deur die larfstadia van die vlieë *Gedoelstia hässleri* en *G. cristata* versoorsaak word wanneer hierdie parasiete vreemde gashere, soos skape en beeste binnedring. In hulle natuurlike gashere, die blouwildebees en hartebees, veroorsaak hierdie parasiete skynbaar slegs geringe letsels. Die ontrafeling van die etiologie van hierdie probleemsiekte het wêreldwye aandag getrek in die veterinêre en entomologiese wetenskappe.

Deur omvattende doelgerigte studies toon hy en sy medewerkers voorts aan dat grootlamsiekte, 'n toestand wat deur verlengde dragtigheid gekenmerk word, deur die plant *Salsola tuberculata,* veroorsaak word.

Twee groot probleme wat gevoelige verliese in die karakoelbedryf van Suidwes-Afrika veroorsaak het, word hierdeur ontmasker. Vandag bestaan daar, danksy die navorsing van dr Basson, metodes vir die behandeling van gedoelstiase, en met korrekte weidingsbeheer en boerdery bestuurspraktyke kan grootlamsiekte grootliks beperk word. Die navorsing op uitpeuloog en grootlamsiekte is ongetwyfeld van die heel belangrikste toegepaste veeartsenykundige werk in Suid-Afrika oor die afgelope 2 dekades. Dat dit grotendeels gedoen is tussen al sy ander pligte as staatsveearts is 'n uitsonderlike prestasie.

Dr Basson het ook 'n intense belangstelling in die siektes van wild getoon. Hiervan getuig die 36 wetenskaplike publikasies oor hierdie onderwerp uit 'n totaal van 71. Sommige van die publikasies is so omvattend dat hulle as tesisse eerder as gewone publikasies beskou kan word. Meeste van dié navorsing is gedoen terwyl hy as patoloog by Onderstepoort se Navorsingsinstituut vir Veeartsenykunde werksaam was. Die werk sluit etlike opnamestudies in waarin 'n enorme hoeveelheid materiaal van bv. bobbejane, olifante, buffels e.a. wildsoorte in samewerking met kollegas op Onderstepoort verwerk is. 'n Verskeidenheid van siektetoestande en patologiese letsels is aangeteken, sommige vir die eerste keer, en etlike nuwe spesies van parasiete en mikro-organismes is beskryf.

Een van die belangrikste aspekte van die navorsingswerk oor wild waarby hy betrokke was, was die waarneming van *Besnoitia* siste in blouwildebeeste en rooibokke. Hierdie waarneming het later tot die suksesvolle gebruik van 'n blouwildebeesstam van hierdie protosoë-parasiet as 'n entstof been besnoitiose by

beeste, of olifantversiekte soos dit in Suid-Afrika bekend staan, gelei. Dit is die eerste keer dat 'n lewendige organisme afkomstig van wild as 'n entstof wat in weefselkultuur gekweek word in huisdiere gebruik is. Dit het nie alleen tot 'n nuwe konsep in die immunisering van huisdiere gelei nie, maar ook die belangrikheid van veeartsenkundige navorsing in wild onderstreep, en tot 'n deurbraak in die bestryding van besnoitiose van beeste aanleiding gegee.

Tydens sy verblyf op Onderstepoort het dr Basson ook 'n besondere bydrae tot die uitbreiding van kennis in verband met die patologie van infeksiesiektes en dierevergiftiging gemaak. Publikasies oor babesiose, hepatosoönose, toksoplasmose, pneumosistikose, nosematose, hartwater, drie-dae-stywesiekte, diamidine vergiftiging in honde, dieldrin vergiftiging en geeldikkop is bewyse hiervan.

Dr Basson is ongetwyfeld dié veterinêre patoloog wat gedurende die afgelope paar dekades die grootste bydraes oor 'n besonder wye veld tot nuwere kennis van veeartsenykunde in Suid-Afrika gelewer het.

Alhoewel dr Basson nou terug is in Suidwes waar hy as staatsveearts cum streekslaboratoriumbeampte te Grootfontein werksaam is, is hy steeds aktief besig met veeartsenykundige navorsing. Tans skenk hy veral aandag aan die vatbaarheid van wildsoorte vir gifplante en die aanvaarbaarheid van gifplante vir wildsbokke, met die oogmerk om moontlik plase wat met gifplante besmet is vir wildboerdery eerder as veeboerdery te gebruik. Hy gee ook heelwat aandag aan opnames oor die verspreiding van spoorelemente in Suidwes-Afrika en ook aan ehrlichiose by waghonde, diere wat tans so 'n belangrike funksie aldaar vervul. Daar is reeds aansienlike vordering met die werk gemaak en verdere wetenskaplike prestasies kan van hierdie veelsydige en energieke wetenskaplike verwag word.

Pieter Arnoldus Basson is op 47-jarige ouderdom inderdaad 'n waardige wenner van die gesogte Senior Kaptein Scott medalje van hierdie vereniging.

BOOK REVIEW — BOEKRESENSIE

INDEX-CATALOGUE OF MEDICAL AND VETERINARY ZOOLOGY

Parasite-Subject Catalogue. Hosts Supplement 21 Part 7
Shirley J. Edwards, Martha W. Hood, Judith H. Shaw, Jane D. Rayburn, Margie D. Kirby, Deborah T. Haufman & Judith A. Zidar

Science and Education Administration United States Department of Agriculture
Washington D.C. 1978
pp xvii 394 Price not quoted

This book is essential for any parasitologist, veterinarian, medical physician, zoologist or student interested in parasites and their hosts. It is very easy to acquire the information you need because the cross indices in this part are so well arranged. For example on p. 118 Dog. See *(Canis familaris)* appears. On referring to *Canis familaris* these entries form a long list from page 60–66.

The authors names arranged alphabetically, the year of publication and the parasites in a brief list are given. The reader then consults Part 1 Authors A–Z of Supplement 21 published at the same time for the complete reference.

I unreservedly recommend this book particularly for students doing seminar or writing a thresis and other workers writing articles.

R.K.R.

OPENING ADDRESS

R.D. BIGALKE*

Mr Chairman, Ladies and Gentleman,

It is my pleasant duty to open this symposium on haemotropic diseases. I may say that I regard this task as a great honour, since the symposium is, in my opinion, undoubtedly, the highlight of this National and International Congress of the South African Veterinary Association.

A glance at the scientific programme of this Congress will confirm my conviction. No fewer than 23 scientific papers, inclusive of the keynote address, will be delivered and the programme is to extend over the best part of three days.

The speakers have all made their mark in the field of haemotropic diseases. Prominent among them is the keynote speaker, the director of the world famous Centre for Tropical Veterinary Medicine at Edinburgh. There are no fewer than 12 other participants, mostly from overseas, from countries beyond our borders. Australia, Israel, Kenya, the Netherlands, the United Kingdom, the U.S.A. and Zimbabwe/Rhodesia are all represented here. In fact, I think I can promise that those of you who will be attending the whole symposium will have the rare privilege of seeing and hearing the élite of the scientists who are actively studying this important branch of veterinary science. You will be getting the latest on haemotropic diseases straight from the horse's mouth, so to speak.

This symposium has been made possible by the sponsorship of Wellcome, Southern Africa, and its concept is the inspiration of the Coopers (South Africa) Veterinary Division of Wellcome, an organization which is also deeply involved in research on this topic.

What, may I ask, is the purpose of this symposium? What do we hope to achieve? Wellcome have not indicated what exactly they had in mind in sponsoring the occasion, but they, like everybody who is concerned with ticks and tick-borne diseases, realize that we in South Africa, as elsewhere in Africa and large parts of Asia, have tremendous problems to solve in this regard. I shall largely confine my attention in this address to the South African situation, which I know more intimately.

Tick-borne diseases and ticks are probably responsible for greater direct losses of lives of stock and losses in production in the world than any other disease entity. It is not possible to give an accurate estimate of these losses in South Africa because the necessary statistics and other basic scientific information are lacking. The South African Bureau of Standards has, however, estimated that tick-borne diseases and ticks cost this country a staggering 70 million rand per annum, and some maintain that this is a conservative figure. This estimate should serve as a tremendous challenge to researchers and decision-makers alike, and if this symposium achieves nothing more than to bring this message home it will have been worthwhile. It would be incorrect to deduce from what I have just said that nothing has been done or is being done about the problem of haemotropic diseases in South Africa. This country is where so much of the pioneering work on ticks and tick-borne diseases has been done, and we can justly acclaim the contributions made by people such as Theiler and Neitz. It is here where blood vaccines against anaplasmosis and heartwater were developed. We have "licked" the tsetse fly, and trypanosomiasis is no longer a problem. East Coast Fever, as Theiler and Neitz knew it, was eradicated more than 20 years ago by our Division of Veterinary Services, largely through the use of dipping and quarantine measures that were enforced by an understaffed organization working under considerable pressure and rather primitive managemental conditions, an achievement in veterinary control and surveillance which is probably unsurpassed in the field of statutory control of diseases.

What, however, have we achieved in South Africa since that illustrious period? A truthful answer is: not nearly enough. Perhaps we have been mesmerized into scientific quiescence by the steady stream of wonder drugs and ixodicides. The fact that parasites are genetically well-equipped to deal with poisons seems to have been largely ignored. Redwater, anaplasmosis and heartwater are probably killing off as many cattle now as they did 20 years ago. It is only relatively recently that the dire need for and importance of detailed basic information on the epizootiology of tick-borne diseases and the ecology of ticks have been realized in this country. We were much too inclined to generalize on the strength of very limited information in the past.

The purpose of this symposium should therefore be, not only to bring us up to date with the latest research results in the field of haemotropic diseases, but also to serve as a forum to take stock of the present situation in this country, to identify the most important bottlenecks in the field of haemotropic diseases and to give guidance on research priorities. This, I am convinced, is why our Chairman, Dr Graeme Thompson, has stressed all along that there must be ample time for discussion during this symposium. I trust I am not being overoptimistic in stating these aims.

The South African farmer is being bombarded from all sides with conflicting advice on how he should control his ticks and tick-borne diseases. The result is confusion which is not in the interests of this country.

We are in need of a realistic comprehensive plan which takes our resources in manpower and funds into consideration and in which, if necessary, the available control measures are integrated. It may well be expedient to have a separate plan for each of the tick-borne diseases which is coördinated with each of the other plans. Separate plans for different ecological regions, depending upon the epizootiology of the diseases concerned, will probably be necessary. Before we shall be in a position to make decisions on the final policy to adopt, however, it is essential in my opinion that we should know much more than we do about various important aspects such as immunology, serology, transmission, epizootiology and pathogenesis of the tick-

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borne diseases in this country. An interim policy, or policies, therefore seems indicated.

Allow me to give you a bird's eye view of the current situation in South Africa regarding bottlenecks in our knowledge of tick-borne diseases and research priorities:

A safe, effective vaccine against *Theileria parva* remains as necessary as ever for East Africa and would be of considerable value further south to protect cattle against *T. lawrencëi*-infection. The relationship between the various *Theileria* spp occurring in southern Africa needs sorting out, and the aetiology and pathogenesis of cerebral theileriosis remains unexplained.

The babesioses, which affect a wide variety of domesticated animals in this country, are undoubtedly our most important haemotropic diseases. Redwater probably accounts for the greatest cattle mortality in this country, and yet it is only relatively recently that the importance of epizootiological research in this country with its diversity of ecosystems has been realized.

A reliable serological test to study the epizootiology of heartwater and the efficacy of immunization against the disease should have absolute priority. The currently used blood vaccine is not only dangerous for older animals but cumbersome to administer and of relatively low infectivity for cattle.

We don't know nearly enough about the epizootiology of anaplasmosis and recent investigations suggest that previous observations on its transmission are misleading. The value of *Anaplasma centrale* as a vaccine against anaplasmosis has apparently been overestimated, whilst its pathogenicity has certainly been underestimated.

In vitro cultivation of the aetiological agents of haemotropic diseases such as heartwater, babesiosis and anaplasmosis is an objective which should receive top research priority in suitably staffed, well-equipped laboratories, preferably by research workers with considerable experience and expertise in this field.

The biology and population dynamics of the wide variety of ixodid tick species which parasitize stock in this country need to be studied in depth so as to enable us to recognize their weak points. This information will be of great value in future campaigns against tick-borne diseases, particularly if strategic dipping is to be made use of or an eradication campaign is to be launched.

By a combination of good fortune and far-sighted extension services, *Bos indicus* breeds of cattle are fashionable and hence plentiful in those areas of South Africa where ticks flourish. The relative resistance of our cattle breeds to infestation with the wide variety of species of ticks which occur here has not been studied in any detail, however, and deserves much more research attention. This information will be invaluable when resistance to acaricides becomes a problem, as it has become in Australia.

Sweating sickness *(Hyalomma truncatum),* Karoo tick paralysis *(Ixodes rubicundus)* and spring lamb paralysis *(Rhipicephalus evertsi)* are important tick-associated diseases of stock in this country which annually exact a considerable toll, but whose aetiology has not even been properly studied.

These then, Ladies and Gentleman, are some of the research priorities and unsolved problems. It is not unlikely that the participants in this symposium will have different views or suggest other approaches. I sincerely hope that this will be the case and that fruitful discussions will follow. Even if the objectives of this symposium, as I see them, are not all realized, progress in the directions I have indicated will be of considerable value to this country.

Finally, Mr Chairman, Ladies and Gentleman, we salute Wellcome Southern Africa for assisting us in this generous and far-sighted fashion.

KEYNOTE ADDRESS

D.W. BROCKLESBY*

It is a special pleasure for me to pay my second visit to South Africa but I was very distressed to learn on my arrival of the recent death of Dr Willi O. Neitz who was a personal friend and a great scientist of whom all South Africans can be proud. His was a name one naturally links with that of Theiler who set the scene for so many of the discoveries later made by Neitz. He would have enjoyed this symposium so much as his work in Brazil, where he had a group of extremely able and enthusiastic post-graduate students, had ensured his continuing interest in the haemoparasitic diseases. It would be nice if we could, in some way, dedicate this meeting to his memory.

My first visit was, I think, in 1962 when I spent ten days with Willi Neitz at Onderstepoort. This was a most enjoyable and educative experience – quite argumentative, true – but all arguments were conducted with restraint and friendship. And this, I have found is, in the main, rather characteristic of the Tick-Borne Diseases Club. We have our differences of course but these rarely lead to acrimony or hatred – in fact I have discovered that an interest in our little animals has formed the basis for many of the closest frienships that I have made – and I'm delighted to see so many old friends in the audience here.

The main reason for my previous visit was to learn all I could from Willi – and also to discuss a taxonomic question – the validity of *Theileria lawrencei* as a species.

In Kenya we had isolated strains of this parasite, from buffalo and from buffalo-infested grazing, and had passaged them with *R. appendiculatus* ticks through cattle. In some of these passage lines changes took place – there was an increase in the size and number of macroschizonts; microschizonts began to appear and, with them, there was an increase in the number of piroplasms in the red cells.

These changes meant that the parasite had changed its character so that it had become indistinguishable from *T. parva.*

Before he would settle down to talk about this Willi spent a morning showing me some slides. I didn't realise this at the time but I think that this was by way of being a little test. I remember that the last slide was of some peculiar bodies in goat red cells that had me stumped as I fiddled about with the condenser (which of course was perfectly in adjustment). Finally a dim bell rang in my head and I muttered "Mukherjeella" – I had passed the examination!

We debated the *lawrencei* question as thoroughly as our then rather superficial knowledge allowed – and came to no particular conclusion except that the buffalo was a reservoir of something nasty that was uncomfortably like East Coast fever. On my way back to Kenya I spent some days in Rhodesia where my view, that there was no such thing as *T. lawrencei,* went down like a lead balloon. They had, after all, spent many years and much effort fighting ECF and had only a few years previously finally declared the country to be free from infection. They were not going to allow some pipsqueak from Kenya tell them that Rhodesian Theileriosis was the same thing as East Coast fever!

If this was the case, they argued, why had ECF not reappeared in Rhodesia? My answer then was that it *could* do so and *would* do so – if only they would allow the parasite to fulfil its destiny by not leaping on outbreaks with such efficiency.

Well – all this is quite a long time ago now – and much work has been carried out on the problem since I left it 15 years ago.

Other isolates have shown the same changes that we observed in Kenya in the 60's and in Kenya they now talk about strains such as "*Theileria lawrencei* – Serengeti Transformed" – the word Transformed being used in the sense that it has changed into *T. parva.*

Serology suggests that *T. parva* and *T. lawrencei* are identical but cross immunity trails in cattle indicate some differences. Dr Uilenberg and Dr Lawrence are going to bring us up-to-date later on and I only want to say that the idea of a trinomial solution seems to me to be a good compromise. But will we ever really get used to saying *"parva-parva"* or *"parva-lawrencei"?*

Of course the other Theilerial species, particularly *T. mutans* and *T. sergenti,* are also causing taxonomic problems and I am looking forward to hearing the later opinions on these. In 1976 I put forward (I hope sufficiently tentatively!) the view that there were four valid species in cattle – transmitted by four different genera of ticks:-

T. parva is transmitted by *Rhipicephalus*
T. annulata is transmitted by *Hyalomma*
T. mutans is transmitted by *Amblyomma*
T. sergenti is transmitted by *Haemaphysalis.*

I have a feeling that this tidy solution will take a beating during the next few days! The situation in Rhodesia is particularly intriguing with one type of theileriosis being maintained in cattle and the other dependent on the presence of buffalo. Maybe iso-enzyme studies or the use of monoclonal antibodies will eventually resolve the problem – but we should be wary of such sophisticated methods.

A keynote speaker is in a bit of quandary. He must be careful not to pre-empt any of the subsequent speakers (and this is a bit of a temptation as he has had a good look at the abstracts) but he must also try to avoid being too nostalgic or anecdotal – and he must *not* go into too much detail. I suppose that my function is to act as a fairly light-hearted and light-weight curtain raiser. At any rate these notions provide me with complete freedom to say whatever I choose.

Well – it seems that, in harking back to the early buffalo days, I had embarked upon the boring and turgid subject of Taxonomy. Taxonomy really is a horrid nuisance – but of course it is an absolutely necessary nuisance. Without a firm and well-based classification and nomenclature we simply cannot talk to each other.

Amongst the parasites we are to discuss during the next two and a half days perhaps the Piroplasms pre-

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sent us with the most awkward problems and I would like, quite briefly, to refer to a few of these.

At a Conference held in Edinburgh in 1976 I made a plea for the retention of the genus *Cytauxzoon,* species of which have been found in a number of African antelopes (to say nothing of Egyptian fish and American cats). This was in reaction to Professor Levine who dictatorially sank the genus in 1971 and merged it with *Theileria:* he did this for reasons which I found extraordinary and completely unacceptable.

Very recently (July 1979) Grootenhuis and his colleagues in Kenya have published some work which throws new light on this subject.

They were able to set up some experimental infections in captive eland by the use of a tick-derived stabilate.

From these infections they established tissue cultures: most of these behaved like normal *Theileria* infected cells but two isolates were peculiar in that they grew as monolayers and the parasitized cells were like macrophages. Within this monolayer large multinucleate cells were present and these were parasitised by large collections of schizonts or cytomeres. They were, in fact, very similar to the forms that I saw in a dead eland in 1960.

The authors did not, however, conclude that their eland was infected with two parasites but they felt that this was unlikely because the parasite had been passaged twice by ticks and once by syringe before the cell culture isolations were made. What is more, they showed, with the IFA test, that the *Theilera*-like cultures were antigenically similar to the *Cytauxzoon*-like cultures.

This conclusion, which is really the same as that of Levine, but for real *reasons* based on actual observation of the parasites, seems sensible to me and I agree that we should drop the name *Cytauxzoon* for the moment and place the known species with *Theileria.* The eland parasite becomes *Theileria taurotragi* and it could prove to be a very useful research tool – as a negative to other *Theileria* species in serological investigations.

Another genus that has recently bit the dust is *Haematoxenus* – "the veiled parasites of Uilenberg". We were pleased to publish this elegant ultra-structural work in Research in Veterinary Science last year.

These veils had been an enigma since they were first seen by Uilenberg in 1964 and this latest work of his clearly showed that they are not a part of the parasite – they were shown to be crystalline and to stain with benzidine like haemoglobin.

This means that the genus *Haematoxenus* must also be placed on the scrap heap and that the parasites must be put into the now rapidly expanding genus *Theilera* – so the bovine parasite becomes *Theileria velifera* and the sheep one is *Theileria separata.*

So it appears that we are tidying up the Theileriidae and I would be interested to know whether the *Anaplasma* people are prepared to be as vicious with the dubious genus *Paranaplasma*?

Turning now to *Babesia* and related parasites, there has also been a recent tidying operation regarding one particular genus and that is *Nuttallia.* This has been used to encompass the small Babesidae that divide into four to form the well-known "Maltese Cross" forms. There has been a rather low-key debate going on about the validity of this genus for many years but the matter has now been settled, at least as far as the word *"Nuttallia"* is concerned, by some correspondence that took place after Pierce had described a new parasite – a piroplasm that he found in crowned cranes in Chessington Zoo. Dr Coan then wrote to him and pointed out that the genus *Nuttallia* had been set up by a man called Dale in 1898 – for species of North American bivalves! This had been overlooked by Franca in 1909 when he used the word for piroplasms and, even more amazingly (and perhaps reprehensibly), we protozoologists had not noticed this for the next 60 years.

There are various other names that we *could* use for these Maltese Cross forms – such as *Achromaticus, Smithia* or *Nicollia,* but I think that we should, again "for the moment", let them rest in peace in the genus *Babesia.*

In cattle the *Babesia* scene seems fairly quiet just now with most authorities agreeing that there are four species and that these are *Babesia bigemina* and *B. major* – the two large ones, and *Babesia bovis* and *B. divergens* – the two small ones.

Serological results support this and the only awkward one is *B. berbera* which seems to be a strain of *B. bovis* with some strange habits.

There are two trivial genera waiting for the axe-man – *Echinozoon,* which occurred, once, in the rock hyrax – and *Entopolypoides* – a parasite of monkeys. Both these must remain on the books until more work is done. *Anthemosoma* a parasite of the spiny mouse, is another odd-ball – it seems to be a link between the piroplasms and the malaria parasites, with intra-erythrocytic schizonts producing from eight to sixteen merozoites – but no pigment.

Well – that would seem to be about enough on taxonomy and I would now like to mention a few topics that have caught my fancy recently. I hope that those of you who find that I fail to give your favourite parasite an honourable mention won't be offended – there will be plenty of time for you to put this right during the next two and a half days.

From the programme I note that, out of 22 papers, we have eight on *Babesia,* five each on *Theileria* and *Anaplasma,* two on Heartwater, one on Tick resistance and a paper that I could only categorise as *General* – by Dr Noval in a few minutes.

The piroplasms and anaplasms always seem to be in the limelight but I am particularly pleased to see that we have two papers on heartwater to look forward to.

Heartwater is a disease that has been rather seriously underrated and this was recognised by the FAO consultation that was held in Rome in December 1977. Dr Uilenberg prepared an excellent "state of the game" working paper for that meeting that concluded with a statement of our deficiencies. Since treatment is very often *too late* we must look towards immunization as the only realistic answer – and we have a paper later on by Dr van der Merwe describing the methods she uses successfully in the Northern Transvaal. Probably a real breakthrough will follow on from successful cultivation of the rickettsia, either in lab. animals or in tissue culture. Strains do vary considerably in virulence so maybe we should attempt to trap some mild strains and see whether they will immunise against virulent ones. It may well prove essential to use local strains for immunisation programmes.

"On-Farm" immunisation methods usually employ relatively large quantities of blood that have to be given

intravenously – maybe it would be better to use frozen brain material or perhaps tick derived stabilates of the type used in the infection and treatment method for East Coast fever.

This method, which was based on the early work of Neitz, was developed by the ECF project at Muguga in Kenya. Sadly the FAO component of the project has been terminated – but there is still a strong team there – mainly financed by British Aid – so the work will continue. The team achieved a great deal and Dr Purnell has published a comprehensive review of the work that runs to some 50 pages.

At the outset the group decided that the techniques left to them by the earlier workers were quite hopeless to support any quantitative investigations.

They determined that it was essential to do four things:

1. Obtain suspensions of parasites *from ticks* that would regularly infect cattle by inoculation:
2. Devise reliable freezing methods for such stabilates:
3. Devise methods for the filtration of the stabilates:
4. Grow the parasite in tissue culture *really properly*.

A fifth item of very high priority was the establishment of a simple and reliable serological test.

It is greatly to the credit of that group that they achieved all these objectives – and the techniques they developed have now become standard methods that are used quite widely throughout the interested world.

The infection and treatment method for East Coast fever is very simple:

Ticks are pre-fed for four to five days, usually on rabbits, to mature the parasites in their salivary glands:
They are then ground up in a pestle and mortar with Scottish sand in medium:
After standing in a measuring cylinder for the duration of a long coffee break the supernatent fluid is collected and this is the infective material which can be cryopreserved with glycerol.

These are the famous GUTS – ground up tick stabilates, and they have three main advantages over the old 10-tick challenge:

Firstly – reactions are reproducible
Secondly – infections are always lethal
Thirdly – no ticks are used to transmit (no nasty ear bags, etc.)

The Muguga group have refined the method, which is fully described in a series of papers by Radley *et al* and it is now, and was in 1975, at a stage where it could be used as a practical method for the immunization of cattle.

Of course there are some disadvantages, such as the possibility that a carrier state could be set up and lead to the introduction of alien strains. But none of the disadvantages would seem to me to be very important – certainly they wouldn't make the situation in an endemic area where ECF was rampant ANY WORSE.

In parallel with the work on infection and treatment, Brown and his colleagues made great advances on the tissue culture side and at one time it looked as though they were about to produce a vaccine – much as the Israeli workers had some years before. Duncan Brown is with us, however, and since he will be describing some latter day uses of the tissue culture system, I will say no more on this subject now.

Another piece of work which I find fascinating – and rather horrific – is the attempts made by Irvin *et al* to infect mice with ECF. Beginning with irradiated ordinary Swiss mice they found that inoculations of *T. parva* tissue cultures resulted in the establishment of massive tumours of infected bovine cells. These tumours were invasive – more so than bovine lymphosarcomas – but were eventually rejected. This rejection problem was overcome by the use of irradiated congenitally athymic nude mice and in these animals huge tumours would grow and frequently resulted in the death of the mouse.

The work was started with two objectives –

1. the setting up of a laboratory model system; and
2. the hope that attenuation might occur.

There was a glimmer of hope in both areas.

In some of the mice theilerial piroplasms appeared in their red cells – this must mean that the life cycle was completed – but these experiments were not reproducible. In a single experiment it appeared that attenuation had occurred after six passages in nude mice: the cells were then put back into six cattle and they underwent mild reactions but were *subsequently shown to be immune to a lethal challenge!* The authors rather quaintly suggested that the method "deserves further attention" and this is surely an understatement. But as far as I know the work has not been followed up.

When he returned to Compton, Irvin continued to try and find ways of persuading parasites to grow in unusual cells. He used cell fusion techniques – and he tried to grow *Babesia* parasites in haemoglobin-producing leukaemia cells. He also tried to use parasites to complement enzyme deficiency that had been induced in cell lines and, in connection with this work, he made some studies on the biochemical needs of *Babesia* parasites. Using four species, *B. rodhaini* and *B. microti* from mice, and *B. divergens* and *B. major* from cattle, he maintained the parasites in Eagles minimal essential medium and added different radioactive purines and pyrimidines. The parasites selectively took up several purines – and had a particular avidity for tritiated hypoxanthine: uptake of this was directly related to the metabolic activity of the parasites. By accident, then, he had come across a possible tube test that could be used to measure the viability of *Babesia* parasites after various insults. This work was recently summarised in a paper presented at a Symposium run by the I.A.E.A. in Vienna and should be studied by anyone who has an interest in reducing the numbers of laboratory animals used in experiments. The method could be used as a primary screen for potential drugs or to measure the effects of different culture media, the effects of irradiation or the effects of different methods of cryopreservation.

Talking of chemotherapy – I am glad to see that we have some papers on this subject as it sometimes gets relegated to the kitchen whilst esoteric and cultivated discussions on immunology and molecular biology take place.

With regulations and legislation becoming so strict in most countries it is amazing to me that any commercial concern can be bothered with anything other than money-spinning tranquilisers and suchlike. We have to be grateful that some companies are still prepared to search for solutions to relatively minor problems. It is, I assure you, a pure coincidence that the two compounds

I am about to mention are developments of Wellcome who paid for me to come to South Africa! Imidocarb is a drug with some very interesting properties as far as *Babesia* parasites are concerned. Firstly, it has very considerable prophylactic powers – this, of course, means that residue problems are bound to occur. Wellcome, to their great credit, have carried out the necessary and expensive defensive research and the drug has now been re-introduced. At quite an early stage it was found that it was not absorbed through the gut – so it would be quite alright to eat meat heavily laced with Imidocarb. Nevertheless, the law-makers insisted – and so the research had to be done. Apart from its prophylactic effect there is another intriguing piece of work, published by Kuttler and colleagues in 1975, that indicates that infected ticks that feed on Imidocarb-treated cattle lose their infection. I don't know whether this work has been extended or confirmed but, if it is a fair bill, one can envisage the use of Imidocarb in eradication programmes in certain situations.

In 1976 McHardy and his colleagues published a paper in "Nature" that I took the liberty of calling 'poetic justice'. This described the effect of menoctone on East Coast fever and the results were truly amazing. It was 'poetic justice' of course because the Wellcome people had spent years and years vainly looking for a drug against ECF at their specially constructed labs. in Kenya and had eventually abandoned the search. I understand that Menoctone itself was too difficult or costly to synthesise but that they have now developed an analogue which is even better – and more amenable to manufacture. We await the final outcome with great excitement – but I would suggest that we must be patient and not press the researchers with importunate enquiries. This sort of work is laborious and we must leave them alone to get on with it.

In conclusion I would like to refer to a few other pieces of work and perhaps ask some questions. "What about soft ticks" is my first query. It was in 1971 that Gunders discovered the piroplasm in *Psammomys obesus* that was later shown to be transmitted by *Ornithodorus erraticus* and I wonder if anyone has found any more soft ticks to be vectors of piroplasms!

What about blood sucking nematodes as potential vectors? Did "our" parasites *originate* in arthropods – or did they become selected to use arthropods as vectors? Maybe nematodes have some parasites that could adapt themselves to mammalian hosts?

The ELISA test has been under study for some years now; is it about to take over from the IFA test as the best buy? Should we confine serological tests to the laboratory (where they are useful for taxonomic purposes and for the elimination of previously exposed animals from experiments) – or continue to use them for survey purposes? Perhaps it is silly to ask such *general* questions – but it does seem to me that serological tests very often only confirm what we already *know* – that there was "an awful lot of infection about".

Finally, I would like to paraphrase my old friend, Roger Purnell, who recently wrote an interesting article entitled "Tick-borne diseases of cattle – a case for pragmatism?" His message poses the question "Are we being too clever?" Certainly there are methods available for the control of most of the diseases that we are about to discuss and perhaps we should be more energetic in the use of the remedies that we have to hand and not spend so much time in the search for a Holy Grail-type of vaccine for everything. I'm sure that Roger Purnell won't mind if I close with a little gentle teasing: I know he has presented excellent papers recently at conferences in Mexico City and Vienna – and here he is in Johannesburg. In the paper I have just mentioned he suggests that more effort should be made to implement current research findings "possibly" and I quote "at the expense of reducing the infinite round of conferences designed in many cases to reiterate the recommendations of previous ones".

TICK INFESTATIONS AND TICK-BORNE DISEASES IN ZIMBABWE RHODESIA

R.A.I. NORVAL

ABSTRACT: Norval R.A.I. **Tick infestations and tick-borne diseases in Zimbabwe Rhodesia,** *Journal of the South African Veterinary Association* (1979) **50** No. 4 289-292 (En) Veterinary Research Laboratory, P.O. Box 8101, Causeway, Salisbury, Zimbabwe Rhodesia.

The distribution of tick species in Zimbabwe Rhodesia is significantly influenced by land utilization practices. The most commonly occurring species in the over-grazed tribal areas is *Boophilus decoloratus*. By contrast, well-managed commercial farms and ranches support a wide range of tick species, the most important of which is *Rhipicephalus appendiculatus*. Until recently tick-borne diseases of cattle were efficiently controlled through dipping. The low incidence of disease was an important factor contributing to over-grazing in tribal areas.

Between 1973 and 1978 political unrest resulted in a collapse of the dipping service in tribal areas. Populations of *B. decoloratus* built up and outbreaks of babesiosis and anaplasmosis occurred, normally between one and three years after the cessation of dipping. Reduced grazing pressure after the initial disease waves resulted in increased grass cover, allowing species such as *R. appendiculatus* and *Amblyomma hebraeum* to become re-established. Outbreaks of theileriosis and heartwater usually followed, decimating the already depleted herds. The heavy cattle mortality resulted from lack of immunity to tick-borne diseases, as a result of efficient disease control through dipping in previous years.

INTRODUCTION

Relative to its size, Zimbabwe Rhodesia supports the largest number of cattle of any country on the African continent. The growth of the national herd, from 66 000 in 1905 to over 6 000 000 by 1975, was made possible by implementation of efficient disease control measures.

In areas free from tsetse fly, ticks and tick-borne diseases presented the most serious threat to livestock production. This was realized in the early part of the century, when East Coast fever *(Theileria parva)* was introduced from Tanzania and devastated existing cattle herds. A policy of compulsory dipping of cattle in acaricides was introduced in 1914 for the control of East Coast fever and proved extremely successful in controlling and finally eradicating the disease by 1954. As a result of compulsory dipping other tick-borne diseases such as redwater *(Babesia bigemina),* heartwater *(Cowdria ruminantium),* Rhodesian theileriosis *(Theileria lawrencei)* and gallsickness *(Anaplasma marginale)* were brought under effective control and eradicated from large areas.

The disease-free conditions created as a result of effective tick control had a major impact on the development and ecology of rural areas. A thriving commercial cattle industry developed on privately owned farms and ranches, while in the Tribal Trust Lands (TTLs) cattle numbers increased to a point where they were limited only by the carrying capacity of the land. As a result of over-grazing in TTLs, environmental conditions became unsuitable for the survival of most two- and three-host tick species[3]. The most commonly occurring tick in TTLs was the one-host species, *Boophilus decoloratus,* and even this was generally efficiently controlled by dipping. Through pasture management and controlled stocking rates few commercial farms were over-grazed and they supported a wide variety of ticks, including two- and three-host species such as *Rhipicephalus evertsi evertsi, Rhipicephalus appendiculatus* and *Amblyomma hebraeum*. Tick-borne diseases were thus more prevalent on commercial farms than in the TTLs.

In 1973 TTLs in the north-east of the country were infiltrated by insurgents, and the dipping of cattle ceased. As the conflict escalated between 1973 and 1978 dipping was disrupted in more and more areas, to the extent that by the end of 1978 only approximately 20 % of the 3,2 million tribal cattle were being regularly dipped. The break-down of dipping triggered an increasingly complex sequence of events involving a succession of tick species and tick-borne diseases.

The early stages of the break-down of dipping and its effects on ticks and tick-borne diseases in the TTLs have already been described[4,5]. This paper presents an up-to-date and more complete summary of events.

THE BREAK-DOWN OF DIPPING

As a result of the lack of apparent disease threat to their cattle and a reluctance to pay dip fees, many tribal people in Zimbabwe Rhodesia resented compulsory dipping. For this and other reasons, including intimidation, administrative difficulties and impaired freedom of movement, effective dipping ceased in almost all TTLs to which the war spread (Fig. 1); that is, in the

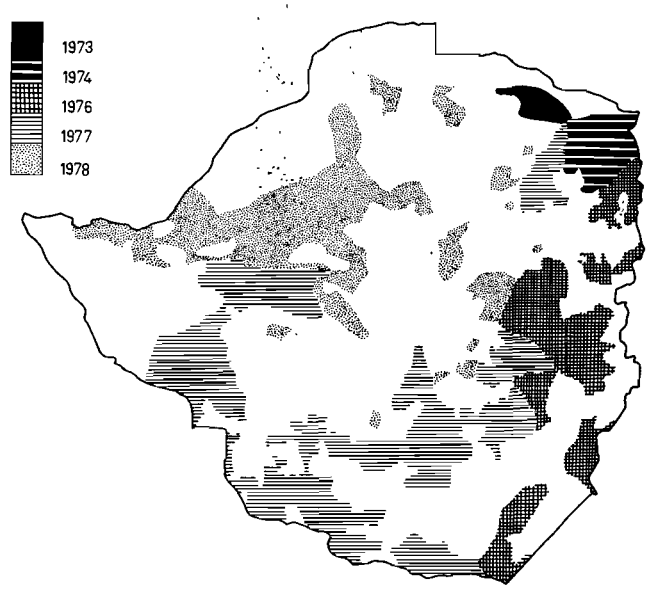

Fig. 1. Map of Zimbabwe Rhodesia indicating areas in which the dipping of cattle has been disrupted, and the year in which this occurred.

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north-east in 1973/74, along the eastern border in 1976, in the south-west and western areas in 1977 and in the north-west and central areas in 1978. By the end of 1978 approximately 1400 (80 %) of the 1760 dip tanks situated in the TTLs had ceased to operate. In some areas dipping has re-started after interruptions of varying lengths of time.

TICK INFESTATIONS

The cessation of dipping has seldom resulted in rapid increases in the levels of tick infestation. This has probably been due to the almost tick-free conditions which existed in the TTLs prior to the break-down of dipping. *B. decoloratus* has almost always been the first tick to occur in large numbers, usually between 12 and 36 months after dipping has ceased. The build up of *B. decoloratus* has been most rapid in the higher rainfall eastern parts of the country. At a local level the degree of over-grazing, the previous history of tick control and the proximity to other tick-infested areas have also been of importance in determining the rate of increase of *B. decoloratus*.

The outbreaks of tick-borne disease which have generally occurred after *B. decoloratus* has become abundant have reduced cattle numbers and hence grazing pressure on the vegetation. Subsequent increases in grass and shrub cover have increased the favourability of the environment for tick survival and have allowed two- and three-host species such as *R.e. evertsi, Hyalomma marginatum rufipes, Hyalomma truncatum, R. appendiculatus, Rhipicephalus simus* and *A. hebraeum* to become established and/or increase in abundance. The spread of tick species into previously uninfested areas appears to have occurred largely as a result of unauthorized movements of tick-infested cattle. In TTLs bordering game reserves, wild ungulates have probably also been of importance.

DISEASES

Gallsickness (Anaplasmosis)

Gallsickness, which is transmitted by *B. decoloratus,* has been recorded in almost all areas in which there has been no dipping for 1–2 years (Fig. 2). Cattle deaths as a result of the disease have occurred throughout the year, but in greatest numbers during the summer months when tick infestations have been heaviest. Although gallsickness has probably caused many thousands of cattle deaths throughout the TTLs, it has been found to occur sporadically and has not been recorded as the cause of heavy mortality in any single area.

Redwater (Babesiosis)

Redwater *(B. bigemina),* which is also transmitted by *B. decoloratus,* has been recorded throughout the TTLs in the eastern half of the country (Fig. 3). Outbreaks of the disease have normally occurred in the second rainy season after the cessation of dipping and have frequently resulted in heavy mortality. Why redwater has not been recorded in the western half of the country is not understood, particularly as *B. decoloratus* is present throughout the area. The answer may simply be that the disease had been eradicated as a result of dipping and has not been re-introduced.

Fig. 2. Map of Zimbabwe Rhodesia indicating areas in which anaplasmosis *(Anaplasma marginale)* has been recorded subsequent to the cessation of dipping. ? = unconfirmed.

Fig. 3. Map of Zimbabwe Rhodesia indicating areas in which redwater *(Babesia bigemina)* has been recorded subsequent to the cessation of dipping. ? = unconfirmed.

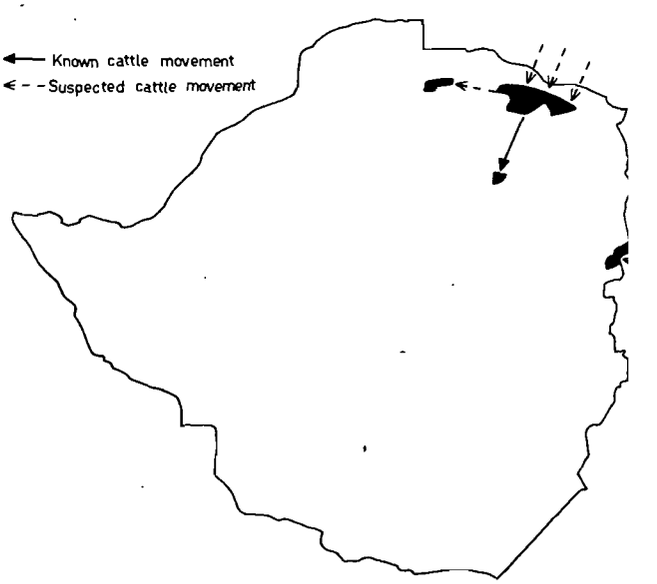

Fig. 4. Map of Zimbabwe Rhodesia indicating the known distributior of *Boophilus microplus* in August 1979.

Jooste[2] reported that *B. microplus,* the known vector of *B. bovis* in southern Africa[1], was absent from Zimbabwe Rhodesia. The species was, however, known to be present in Mozambique. Since 1975 *B. microplus* has been recorded from some TTLs close to the Mozambique border, probably having been introduced with cattle brought into the country by refugees. As a result of the lack of dipping in these border TTLs, the species has become well established and is beginning to spread deeper into the country (Fig. 4). Associated *B. bovis* infections have been confirmed.

Heartwater (Cowdriosis)

A. hebraeum is the principal vector of heartwater in Zimbabwe Rhodesia[6]. In the absence of large numbers of wild ungulates the tick is easily controlled by dipping and by the 1950s it had been virtually eradicated from the TTLs. By 1976 heartwater persisted in only a few ranching areas in the south of the country (Fig. 5) where *A. hebraeum* was maintained in association with wild ungulates. With the break-down of dipping in the southern TTLs there has been a rapid spread of *A. hebraeum* and heartwater from adjoining ranching areas into the TTLs. This has been followed by mortality in cattle, sheep and goats, which has increased as *A. hebraeum* has increased in abundance. Although accurate figures are unavailable, it is clear that heartwater has caused very heavy cattle mortality in some areas. Most heartwater deaths have been reported during the summer months when infestations of adults of *A. hebraeum* have been heavy.

The ability of *A. hebraeum* to spread rapidly apparently results from the lack of host specificity of the immature stages, which frequently parasitize birds and other small vertebrates which are not restricted by fences[5]. After initial increases in the abundance of *A. hebraeum* in the absence of dipping, the spread of heartwater has proceeded extremely rapidly. Unless dipping in the tribal areas is restored in the near future it is clear that heartwater will become an ever more serious problem in Zimbabwe Rhodesia.

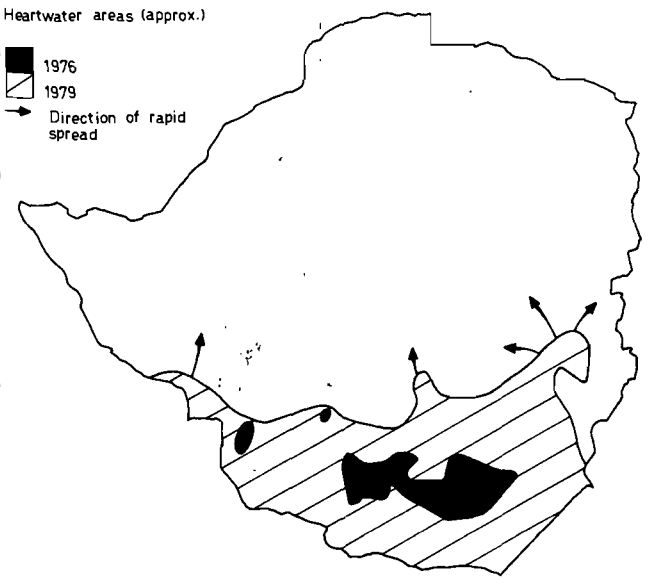

Fig. 5. Map indicating the spread of heartwater *(Cowdria ruminantium)* in Zimbabwe Rhodesia between 1976 and 1979.

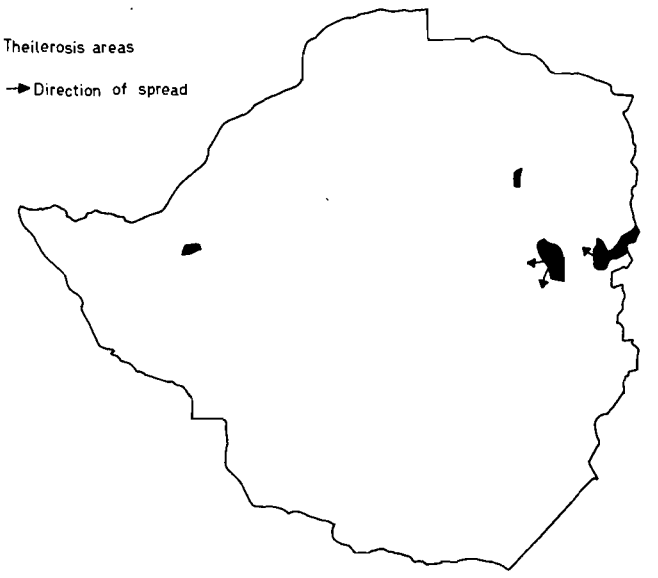

Fig. 6. Map of Zimbabwe Rhodesia indicating areas in which Rhodesian theileriosis *(Theileria lawrencei)* has been recorded subsequent to the cessation of dipping.

Theileriosis

Theileriosis was absent from the TTLs prior to the break-down of dipping, as a result of the absence of *R. appendiculatus.* Subsequently, heavy infestations of the tick have only been observed after outbreaks of redwater and gallsickness have reduced cattle numbers and vegetation cover has increased. To date, theileriosis has been recorded in only a few TTLs (Fig. 6), with serious outbreaks having occurred only in the high rainfall eastern border area where *R. appendiculatus* has become well established. The single outbreak in the north-west of the country occurred after undipped cattle had been in contact with buffalo, but there has been no subsequent transmission to other cattle. Of all the tick-borne diseases currently recorded in TTLs, theileriosis appears to have caused the highest proportional mortality in cattle. It has also been observed that there has been a high mortality in calves born to cows which had apparently survived the disease.

Other tick-borne diseases

Spirochaetosis *(Borrelia theileri)* and ehrlichiosis *(Ehrlichia bovis),* both mildly pathogenic, have also been recorded from TTLs where dipping has broken down. Both diseases were detected in experimental cattle on which field collected ticks had been fed. Spirochaetosis was transmitted by *B. decoloratus,* and ehrlichiosis was transmitted by adults of *R. appendiculatus* (collected as engorged nymphs).

CATTLE DEATHS

Norval[5] estimated that more than 300 000 cattle had died of tick-borne diseases in the TTLs between 1974 and April 1978. In the 1978/79 rainy season mortality was considerably higher than in previous years and although accurate estimates have been impossible to obtain it is reasonable to state that the total number of cattle deaths must be approaching one million.

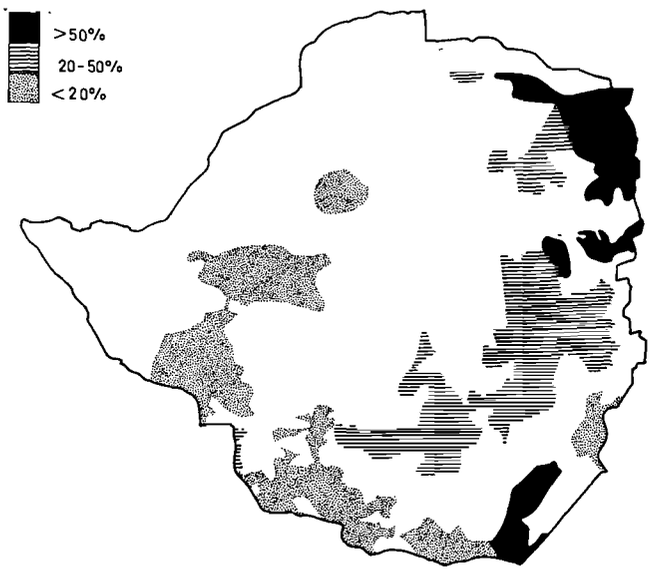

Fig. 7. Map of Zimbabwe Rhodesia indicating cattle mortality, by August 1979, in areas where dipping has been disrupted.

Despite the lack of accurate data it has been possible to determine the general pattern of cattle mortality in different areas (Fig. 7). A mortality rate of over 50 % has occurred in the north-east, east and south-east of the country where dipping has not taken place for 3 years or longer. In a large area, mostly in the eastern half of the country, where dipping has not occurred for 2–3 years, the estimated mortality is 20–50 %. In most of the west and south-west of the country, where there has been no dipping for 1–2 years, and in one area on the eastern border, where dipping was re-introduced prior to any serious disease outbreak, the mortality has been less than 20 %. The percentage mortality in cattle has been highest where redwater has been followed by theileriosis or heartwater. In the Holdenby TTL (eastern border), where redwater and theileriosis have been recorded, the estimated cattle mortality has been 95 %. The numbers of cattle deaths have also been high where *B. microplus* has been present, presumably as a result of the combined effects of *B. bigemina* and *B. bovis* infections.

DISCUSSION

The break-down of dipping in tribal areas in Zimbabwe Rhodesia has presented a unique opportunity to study the dynamic relationships which exist between ticks, their hosts, the diseases they transmit and the environment, and how these relationships are influenced by the use of acaricides.

The heavy cattle mortality which has followed the cessation of dipping has undoubtedly resulted from a lack of immunity to tick-borne diseases within the cattle population. This unstable situation has arisen as a result of the suppression or eradication of the tick vectors by dipping and demonstrates the inherent danger of total reliance on dipping for the control of tick-borne diseases. If catastrophies such as the present one in Zimbabwe Rhodesia are to be averted, it is obvious that more emphasis must be placed on immunizing cattle against tick-borne diseases.

Until effective vaccines are available for all of the tick-borne diseases occurring in a given area, however, dipping must remain the principal weapon in any disease control strategy. In the case of Zimbabwe Rhodesia the lack of a theileriosis vaccine will make it necessary to return to rigid dipping regimes if profitable cattle production is to continue. In any event, some degree of tick control must be maintained if healthy and productive cattle are to be raised, particularly in the higher rainfall areas where tick loads become extremely heavy in the absence of dipping.

ACKNOWLEDGEMENTS

The information presented in this paper was obtained as a result of the co-operation of the field staff of the Department of Veterinary Services, the Department of Agricultural Development and the Zimbabwe Rhodesian Security Forces, and is a tribute to their bravery and dedication. I thank Dr J.A. Lawrence of the Veterinary Research Laboratory for constructive criticism of the manuscript.

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OBSERVATIONS ON THE PATHOGENESIS OF ANAPLASMOSIS IN CATTLE WITH PARTICULAR REFERENCE TO NUTRITION, BREED AND AGE

A.J. WILSON*

ABSTRACT: Wilson A.J.; **Observations on the pathogenesis of anaplasmosis in cattle with particular reference to nutrition, breed and age.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 293-295 (En) Oonoonba Veterinary Laboratory, P.O. Box 1085, Townsville 4810, Australia.

Experiments are described to examine the pathogenesis of anaplasmosis in *Bos indicus* cattle with particular emphasis on nutrition, breed and age. The disease seemed to be less severe in animals under a poor plane of nutrition. Breed was shown to have little effect and there was an age resistance. Natural transmission in *Bos indicus* calves occurred with ease in endemic areas. This indicated that the introduction of *Bos indicus* cattle should not adversely affect enzootic stability.

INTRODUCTION

Anaplasmosis in cattle caused by *Anaplasma marginale* is widespread throughout northern Australia in the area infested by *Boophilus microplus*[13 14]. There has been a massive infusion of *Bos indicus* animals into this area in recent years to combat the effects of heat, drought and ticks and to increase grass utilisation[3 8 11 18]. This dynamic biological experiment has largely been successful[2].

Experiments are described to examine the impact of the introduction of *Bos indicus* cattle on some aspects of the pathogenesis and epidemiology of anaplasmosis.

MATERIALS AND METHODS

Experimental design

Four different experiments were conducted as summarised in Table 1. LN is defined as induced non specific decreased consumption leading to a fasting animal. This was achieved by feeding 1MCal ME/80 kg body weight/day leading to a body weight decrease of approximately 5 kg per week. HN is defined as a diet leading to an increase of body weight of 2–3 kg/week.

The animals of Experiment 1 were fed their respective diets for 8 weeks prior to and for 12 weeks after infection. All animals in the other experiments were fed HN. The parameters and sampling intervals used to measure the pathogenesis of anaplasmosis in all experiments are summarised in Table 2.

Good tick control was undertaken in Experiment 4 by regular dipping and pasture rotation.

Table 2: THE MEASUREMENTS USED TO EXAMINE THE PATHOGENESIS OF ANAPLASMOSIS IN THE FOUR EXPERIMENTS

Experiment	Measurements	Sampling interval
1	Body weight change	weekly
	Packed cell volume (PCV)	twice weekly
	Total red cell count	twice weekly
	Haemoglobin concentration	twice weekly
	Mean corpuscular volume	twice weekly
	Erythrocyte sedimentation	twice weekly
	Reticulocyte count	twice weekly
	Parasitaemia	twice weekly
	Transaminase levels	twice weekly
	Humoral antibody response	weekly
	Plasma protein level	weekly
2	Humoral antibody response	weekly
3	Body weight change	weekly
	PCV	twice weekly
	Parasitaemia	twice weekly
4	Humoral antibody response	monthly
	Parasitaemia	monthly
	Body weight change	monthly
	PCV	monthly

Table 1: A SUMMARY OF THE FOUR EXPERIMENTS CONDUCTED TO EXAMINE ASPECTS OF THE PATHOGENESIS AND EPIDEMIOLOGY OF ANAPLASMOSIS IN *BOS INDICUS* CATTLE

Experiment	Group	Treatment	Number of animals	Type	Age at infection (months)	Infective dose
1	1	HN	10	indicus	27	10^{10} IV
Effect of	2	LN	10	indicus	27	10^{10} IV
nutrition	3	HN	10	indicus	27	nil
	4	LN	10	indicus	27	nil
2	1	HN	9	taurus	27	10^{10} IV
Breed	2	HN	18	indicus	27	10^{10} IV
	3	HN	9	taurus	48	10^{10} IV
	4	HN	18	indicus	48	10^{10} IV
3	1	HN	18	indicus	6	10^{10} IV
Age	2	HN	18	indicus	27	10^{10} IV
	3	HN	18	indicus	48	10^{10} IV
4	1	HN; 1–2 in	80	indicus	0–12	Natural
	2	wet tropics;	80	indicus	0–12	challenge
	3	3 in dry tropics	120	indicus	0–12	

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Parasites

a. Derivatives of stabilate Oonoonba-1[19] were used in splenectomised calves in Experiments 1 to 3.
b. Natural challenge in two endemic areas in north Queensland were used in Experiment 4. One area was in the dry tropics and the other in the wet tropics.

Experimental animals

Animals in Experiments 1–3 were obtained from properties which were free of *B. microplus*. The *Bos indicus* type cattle contained a 50–75 % Brahman component. On arrival at the laboratory, the animals were dipped and examined for intestinal helminths and blood parasites by faecal and thin film examinations respectively. Sera from all animals were examined for humoral antibodies to *A. marginale* by the complement fixation (CFT)[12], indirect fluorescent antibody[15] and card agglutination[1] tests. The animals were maintained in a tick free area using the methods described by Johnston and Tammemagi[5]. The animals in Experiment 4 were *Bos indicus* calves born from dams in the two endemic areas.

Serology

A micro-CFT, similar to that described by Martin and Ritchie[10] was used to detect humoral antibodies to *A. marginale* in the animals of all experiments.

Statistics

All data from each animal was entered on to computer files for subsequent analysis of variance.

RESULTS

Experiment 1

Anaplasmosis was more severe in the animals fed on the higher plane of nutrition as judged by body weight change, loss of erythrocytes, parasitaemia, reticulocyte count, mean corpuscular volume and SGOT enzyme levels. There was no significant differences between the groups with respect to humoral antibody response, plasma protein levels and SGPT enzyme levels.

Experiment 2

No significant differences in the development of anaplasmosis in the different groups of *Bos indicus* and *Bos taurus* animals were observed as judged by the parameters used.

Experiment 3

Anaplasmosis was less severe in the 6 month old animals as compared with the 27 and 48 month old ones as judged by PCV fall, parasitaemia and body weight change.

Experiment 4

No obvious clinical disease was detected in any of the animals. Significant falls in PCV were observed in a few calves. Anaplasmosis developed in the majority of animals by 12 months of age as judged by a positive reaction in the micro-CFT.

DISCUSSION

Plane of nutrition does seem to play a role in the development of anaplasmosis in cattle. The reason why animals under a low plane of nutrition had a less severe disease is uncertain. Much information is required on the *in vitro* growth characteristics of *A. marginale*. However, it is known that their amino acid requirement is high and it can only be presumed that there is a deficiency of an essential amino acid in the mal-nourished animal. Reviews on the role of nutrition in disease are given in Sprunt and Flanigan[17] and Hudson et al[4].

The view that *Bos indicus* cattle are more resistant than *Bos taurus* should be accepted with caution as groups of pure *Bos indicus* and splenectomised calves were not examined. Splenectomised calves are considered to be the most sensitive monitor to detect breed differences to babesiosis[6]. Nevertheless these results agree with those of Seifert[16]. Lohr et al[9] considered that *Bos indicus* animals were more resistant than *Bos taurus;* however age resistance could explain their results. Vaccine requirement in *Bos indicus* animals should therefore not be reduced.

The age resistance in *Bos indicus* to anaplasmosis was expected in view of the established resistance in *Bos taurus* animals[7]. This finding together with the ease by which anaplasmosis was transmitted by *B. microplus* to *Bos indicus* calves under good management (Experiment 4) indicates that enzootic stability should be easily achieved in endemic areas. It is of interest that *B. microplus* seems to be an extremely efficient vector of *A. marginale* even in cattle with very low tick numbers.

ACKNOWLEDGEMENTS

I would like to acknowledge my colleagues who assisted and carried out some of this work, in particular Professor R.S.F. Campbell, Messrs S.A. Ajayi, R. Parker and I. Paul.

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IXODICIDAL RESISTANCE IN *BOOPHILUS MICROPLUS* (CANESTRINI) IN THE REPUBLIC OF SOUTH AFRICA AND TRANSKEI

J.A.F. BAKER, JANET O. JORDAAN and WENDY D. ROBERTSON

ABSTRACT: Baker J.A.F.; Jordaan, Janet O.; Robertson, Wendy D. **Ixodicidal Resistance in *Boophilus microplus* (Canestrini) in the Republic of South Africa and Transkei.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 296-301 (En) Kwanyanga Res. Stn, Coopers (SA) (Pty) Ltd, P.O. Box 5034, Greenfields 5208, Cape Province, Rep. of South Africa.

A series of *in vitro* trials using unfed larvae and fully fed adult ticks confirmed ixodicidal resistance in the one-host Pantropical Blue Tick, *Boophilus microplus* (Canestrini). Fifty-seven of 64 field isolates were resistant to arsenic; 10 of 56 were resistant to toxaphene; 1 of 5 were resistant to lindane; 3 of 5 were resistant to dieldrin; 3 of 19 were resistant to DDT and 8 of 55 were resistant to the organophosphorus ixodicide, dioxathion.

One of the field isolates resistant to dioxathion was also highly resistant to the carbamate, carbaryl, and to the organophosphorus ixodicides benoxophos and diazinon. A second was resistant to the organophosphorus ixodicides benoxophos, diazinon, carbophenothion, dicrotophos, ethion, fenitrothion and quintiofos. Low levels of resistance, less than 3X, were shown to chlorfenvinphos and coumaphos. No resistance was shown to chlorpyrifos, bromophos ethyl or the diamidine ixodicide, amitraz.

In hand-spraying trials no variation in the susceptibility of an organophosphorus resistant strain or the susceptible laboratory strain to amitraz was observed.

This is the first recorded resistance to ixodicides by *B. microplus* in Africa.

INTRODUCTION

Although the Pantropical Blue tick *Boophilus microplus* (Canestrini) has been known to exist in Eastern and Southern Africa since early in the present century[2], no resistance to ixodicides by this tick species for these regions has been recorded. In the Republic of South Africa and Transkei the distribution[3] of *B. microplus* is shown to coincide closely with that in which, to a greater or lesser extent, tick control by chemical means has been practised continuously for 50 years or more. Under these conditions some selection for resistance by *B. microplus* to these chemicals might have been expected to occur. In the course of a survey of the susceptibility of ticks to ixodicides during the years 1973–1978, however, only small numbers of samples of *B. microplus* were received. The resistance status of this tick species to ixodicides thus remained largely unknown. Subsequently, in the spring of 1978, a report of a failure to successfully control field infestations of *B. microplus* from the Amatikulu Dip Tank Area, KwaZulu, using the organophosphorus ixodicide dioxathion, prompted an intensive collection campaign of this tick species from this and other regions. Significant variations in the response of some of these field isolates to a range of ixodicides was observed.

Both the extent and the degree of this resistance have been investigated further.

METHODS

Comparative laboratory tests on larvae and engorged adult female ticks were undertaken and in addition, a hand-spraying trial on artificially infested calves was carried out. Details of the methods used were:

Unfed larvae

The technique used was that described by Shaw[4] and later modified[5] to include a longer holding period for the larval ticks after treatment. A further modification to this technique was used in this work whereby one operator carried out the test in duplicate from a common reservoir of treated larvae, as compared to two operators conducting tests simultaneously. This was necessary due to the large amount of larval material required to be tested, as a considerable saving in operator time is achieved by this method.

The standard test ixodicides used were arsenic, toxaphene, dioxathion and chlorfenvinphos. Where sufficient larvae were available or where the isolate was considered to be of sufficient interest to warrant further investigation, DDT, lindane or dieldrin were also included.

A comparison of the susceptibility of larval offspring of four strains to a number of different chemicals was also made: (i) the known arsenic resistant but organochlorine, DDT and organophosphorus sensitive Kwanyanga laboratory reference strain, (ii) the Do Little strain from the Stutterheim District, Cape Province, which had a recent history of resistance to toxaphene in the field, (iii) the Amatikulu strain and (iv) the Ongoye 716 strain, both from the Ongoye District, KwaZulu. Both the Amatikulu and Ongoye 716 strains had a recent history of resistance to dioxathion in the field.

Engorged females

The technique used was that of M.D. Matthewson (1979, personal communication) whereby a reproductive index value for each test concentration is determined by probit analysis following the prior calculation of the estimated reproduction of individual ticks using the formula of Drummond *et al*[1].

The larval offspring of Kwanyanga and Amatikulu strains of ticks were fed on the bodies of calves and engorged females were harvested and randomly sorted into batches of 25 ticks. Each batch was weighed and placed in a PVC tube of 45 mm diameter and 80 mm length, one end of which was sealed with nylon mesh having a nominal aperture of 1 mm. The batches were then individually immersed for 10 min in 60 mm diameter glass jars each containing 50 mℓ of a prepared geometric range of ixodicidal concentrations. After immersion the batches were air dried at room temperature, range 20–26,5°C and the ticks individually removed from the tubes and attached dorsally to strips of adhesive tape mounted on 400 mm x 400 mm boards

and placed in an incubator having minimum environment conditions of 25°C and 80 % R.H.

Inhibition of oviposition and mortality were assessed 14 d after treatment and the egg batch from each tick weighed, individually tubed and returned to the incubator. After a further four weeks the hatch rate of the egg batches was determined and scored on a scale of 0–4 points. Utilising these data the estimated reproduction was calculated.

Hand-Spraying Trial

Four 4 month old Jersey calves, individually restrained in elevated cages with slatted wood floors, were infested with approximately 2 000 larvae of the Kwanyanga or the Amatikulu strain of ticks thrice weekly for 4 weeks. Each day for a 2 d period prior to spraying, 50 engorged female ticks dropping from each calf were collected at random from perforated metal trays stationed beneath the floors of the cages and placed individually in tubes in an incubator (minimum environmental conditions, 25°C and 80 % R.H.) to serve as controls. One of the two calves infested with the Kwanyanga strain of ticks was then handsprayed with 12 ℓ of a 0,05 % dioxathion spraywash and the other with 15 ℓ of a 0,025 % amitraz spraywash. These sprayings were repeated for the two calves infested with the Amatikulu strain of ticks. Post-treatment collections of engorged female ticks dropping from the treated calves were made daily for 14 d and incubated as for the control ticks. The viability of egg batches laid by all ticks was recorded.

RESULTS

Unfed larvae

The results expressed as LC 99 (%) values are the product of at least two duplicates for each observation. The 95 % fiducial limits for these values are also shown. The data were analysed by computer using a probit analysis programme.

The susceptibility of the larval offspring of 64 field isolates of *B. microplus* to arsenic, 56 isolates to toxaphene, 19 isolates to DDT, 5 isolates to lindane, 5 isolates to dieldrin, 55 isolates to dioxathion and 41 isolates to chlorfenvinphos are given in Table 1. A range of values at the LC 99 (%) level for field isolates from each region is compared with those obtained for the Kwanyanga reference strain and factors of resistance thus obtained are given.

Isolates considered as arsenic resistant are those having LC 99 (%) values equal to, or greater than that for the Kwanyanga strain; isolates considered as resistant to either toxaphene, lindane or dieldrin are those having LC 99 (%) values equal to, or greater than, those for the Do Little strain; isolates considered as resistant to DDT are those having LC 99 (%) values equal to, or greater than, 0,3 % and isolates considered as dioxathion resistant are those having LC 99 (%) values equal to, or greater than that for the Amatikulu strain.

Fifty-seven of the isolates tested against arsenic showed levels of susceptibility similar to, or less than, that for the Kwanyanga strain. The geographical distribution of all isolates tested against arsenic is illustrated in Fig. 1.

Ten of the isolates tested against toxaphene, 1 of the isolates tested against lindane and 3 of the isolates

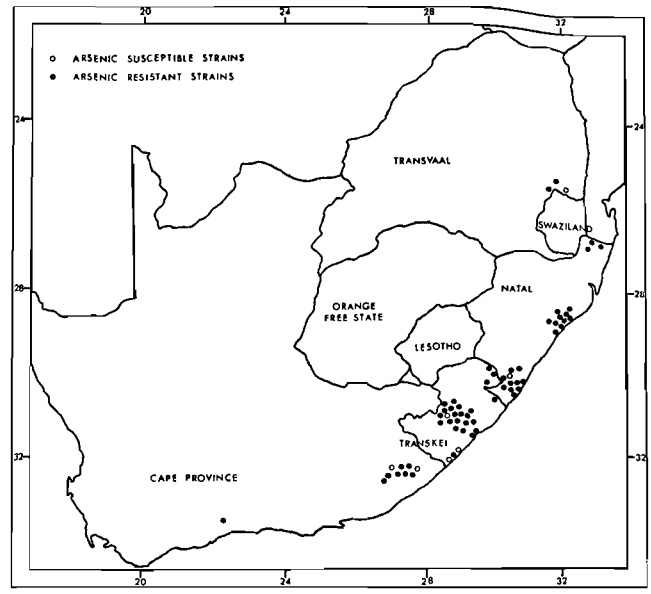

Fig. 1. Geographical distribution of the arsenic susceptible and resistant strains of *Boophilus microplus* recorded in Table 1.

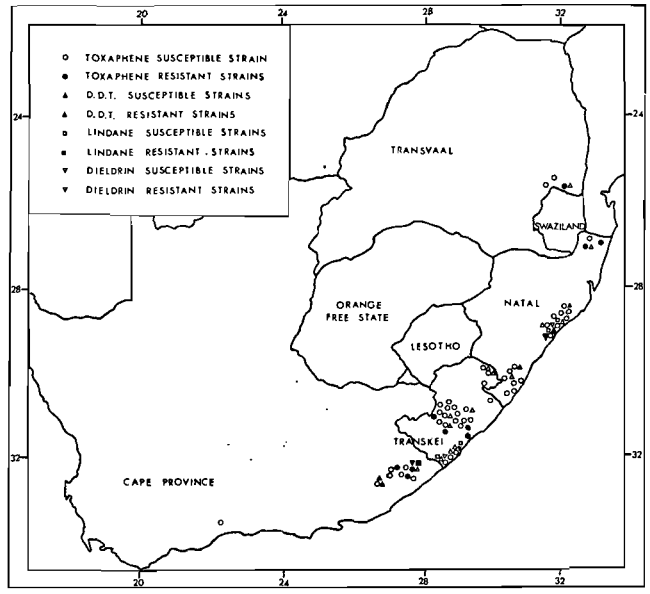

Fig.2. Geographical distribution of the toxaphene, DDT, dieldrin and lindane susceptible and resistant strains of *Boophilus microplus* recorded in Table 1.

tested against dieldrin showed levels of susceptibility similar to, or less than that for the Do Little strain whilst 3 of the isolates tested against DDT had LC 99 (%) values > 0,3. The geographical distribution of all isolates tested against these four chemicals is illustrated in Fig. 2.

Eight of the isolates tested against dioxathion were to varying degrees less susceptible than the Amatikulu strain. None of the isolates tested against chlorfenvinphos showed degrees of susceptibility less than 2,7 x that for the Kwanyanga strain and are, for purposes of control in the field, considered sensitive to this ixodicide. The geographical distribution of all isolates tested against these two chemicals is illustrated in Fig. 3.

Table 1: THE SUSCEPTIBILITY OF 64 FIELD ISOLATES OF *BOOPHILUS MICROPLUS* TO ARSENIC, 56 FIELD ISOLATES TO TOXAPHENE, 19 FIELD ISOLATES TO DDT, 5 FIELD ISOLATES TO LINDANE, 5 FIELD ISOLATES TO DIELDRIN, 55 FIELD ISOLATES TO DIOXATHION AND 41 FIELD ISOLATES TO CHLORFENVINPHOS

Ixodicide	Region	No. of isolates tested	Range of LC(99)%'s	Origin of isolate	LC99(%)	95 % Fiducial Limits Lower	Upper	FOR	No. of isolates considered resistant
Arsenic	Kwanyanga		Reference strain		2,3	1,9	2,8		
	Cape/Ciskei	10	Lowest	Stutterheim	1,9	1,6	2,4	–1,2	8
			Median	Keiskammahoek	3,1	2,5	4,3	1,4	
			Highest	Victoria East	5,5	2,4	>10	2,4	
	Transkei	27	Lowest	Willowvale	2,2	1,9	2,5	–1,0	24
			Median	Tsolo	3,4	1,7	>10	1,5	
			Highest	Ngqeleni	>10	>10	>10	>4,4	
	Natal/KwaZulu	24	Lowest	Vulamehlo	2,1	–	–	–1,1	23
			Median	Ongoye	5,2	2,7	>10	2,3	
			Highest	Inkanyezi	>10	>10	>10	>4,4	
	Transvaal	3	Lowest	Kangwane	1,9	–	–	–1.2	2
			Median	White River	2,3	1,2	>10	1,0	
			Highest	Kangwane	2,8	2,3	3,8	1,2	
Toxaphene	Kwanyanga		Reference strain		0,17	0,11	0,3		
	Cape/Ciskei	10	Lowest	*Outeniqa*	0,039	0,012	>10	–4,4	3
			Median	Keiskammahoek	1,3	0,25	>10	7,6	
			Highest	Keiskammahoek	>10	1,1	>10	>59	
	Transkei	25	Lowest	Bizana	0,015	0,011	0,025	–11	4
			Median	Libode	0,34	0,1	6,7	2,0	
			Highest	Ngqeleni	>10	2,7	>10	>59	
	Natal/Kwazulu	18	Lowest	Ixopo	0,022	–	–	–7,7	2
			Median	Vulamehlo	0,22	0,043	>10	1,3	
			Highest	Ingwavuma	>10	–	–	>59	
	Transvaal	3	Lowest	White River	0,076	0,051	0,13	–2,2	1
			Median	Kangwane	1,1	–	–	6,5	
			Highest	Kangwane	>10	–	–	>59	
DDT	Kwanyanga		Reference strain		0,085	–	–		
	Cape/Ciskei	3	Lowest	Victoria East	0,074	0,059	0,1	–1,1	1
			Median	Stutterheim	0,081	0,025	>10	–1,0	
			Highest	Victoria East	0,47	0,3	0,83	5,7	
	Transkei	8	Lowest	Tsolo	0,02	–	–	–4,2	0
			Median	Tabankulu	0,076	0,057	0,11	–1,1	
			Highest	Willowvale	0,17	0,056	8,6	2,1	
	Natal/KwaZulu	7	Lowest	Vulamehlo	0,015	–	–	–5,5	2
			Median	Ongoye	0,084	0,065	0,15	1,1	
			Highest	Richmond	0,55	0,21	3,6	6,6	
	Transvaal	1		Kangwane	0,12	0,093	0,18	1,4	0
Lindane	Kwangana		Reference strain		0,16	0,11	>10		
	Cape/Ciskei	1		Stutterheim	>10	3,6	>10	>63	1
	Transkei	2	Lowest	Kentani	0,0025	–	–	–64	0
			Highest	Willowvale	0,036	–	–	–4,4	
	Natal/KwaZulu	2	Lowest	Ongoye	0,026	0,0017	>10	–6,2	0
			Highest	Ongoye	0,038	0,0065	>10	–4,2	
Dieldrin	Kwanyanga		Reference strain		0,058	0,015	1,4		
	Cape/Ciskei	1		Stutterheim	>10	6,8	>10	>172	1
	Transkei	2	Highest	Kentani	0,0012	–	–	–48	1
			Lowest	Willowvale	6,9	0,43	>10	>119	
	Natal/KwaZulu	2	Highest	Ongoye	0,15	0,047	1,3	2,6	1
			Lowest	Ongoye	9,3	2,0	>10	160	
Dioxathion	Kwanyanga		Reference strain		0,00087	0,00057	0,0027		
	Cape/Ciskei	10	Highest	Stutterheim	0,0004	0,00036	0,00047	–2,2	2
			Median	Victoria East	0,001	0,0006	0,0034	1,2	
			Highest	Keiskammahoek	0,008	0,0019	>10	9,2	
	Transkei	20	Lowest	Willowvale	0,00042	0,00027	0,0033	2,1	0
			Median	Umzimkulu	0,00096	0,00082	0,0012	1,1	
			Highest	Libode	0,0037	0,00093	>10	4,3	

Table 1 (continued)

Ixodicide	Region	No. of isolates tested	Range of LC(99)%'s	Origin of isolate	LC99(%)	95 % Fiducial Limits Lower	Upper	FOR	No. of isolates considered resistant
Dioxathion	Natal/KwaZulu	22	Lowest	Vulamehlo	0,00059	0,00044	0,016	–1,5	
			Median	Richmond	0,001	0,001	0,0061	2,0	6
			Highest	Ongoye	0,021	0,0095	0,73	24	
	Transvaal	3	Lowest	White River	0,0002	0,0002	0,0003	–4,4	
			Median	Kangwane	0,00073	0,00063	0,00087	–1,2	0
			Highest	Kangwane	0,0021	–	–	2,4	
Chlorfenvinphos	Kwanyanga	Reference strain			0,0025	0,0025	0,015		
	Cape/Ciskei	9	Lowest	Victoria East	0,001	0,0007	0,0033	–2,5	
			Median	Victoria East	0,0023	0,0011	>10	1,1	0
			Highest	Keiskammahoek	0,0068	0,0039	0,024	2,7	
	Transkei	17	Lowest	Tsolo	0,001	0,00089	0,0012	–2,5	
			Median	Umzimkulu	0,0014	0,001	0,0029	–1,8	0
			Highest	Umzimkulu	0,0057	0,0032	0,018	2,3	
	Natal/KwaZulu	12	Lowest	Ingwavuma	0,00078	0,00069	0,00092	–3,2	
			Median	Ixopo	0,0024	0,00093	>10	–1,0	0
			Highest	Inkanyezi	0,0067	0,003	>10	2,7	
	Transvaal	3	Lowest	White River	0,0012	–	–	–2,1	
			Median	Kangwane	0,0021	0,0018	0,0025	–1,2	0
			Highest	Kangwane	0,0038	–	–	1,5	

OR = Factor of resistance determined by comparison with the Kwanyanga strain
– = Fiducial limits not calculated, data heterogeneous

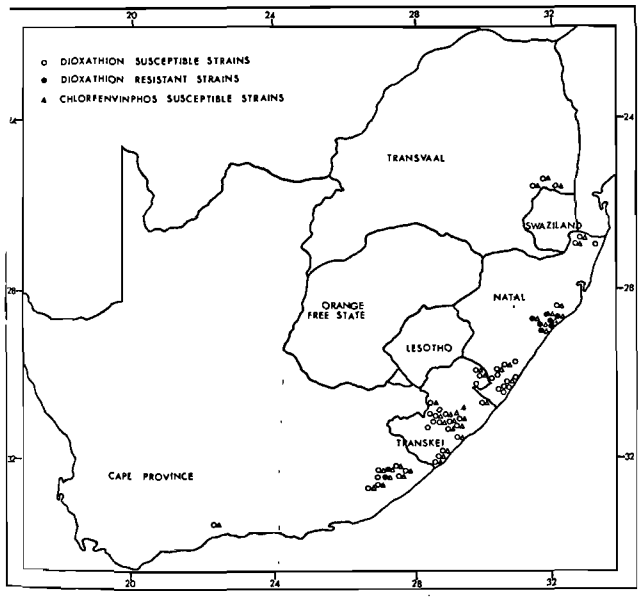

Fig. 3. Geographical distribution of the dioxathion and chlorfenvinphos susceptible and the dioxathion resistant strains of *Boophilus microplus* recorded in Table 1.

The susceptibility of larvae of the Kwanyanga strain to a range of ixodicides is compared to that of larval offspring of strains from Do Little, Amatikulu and Ongoye 716 in Table 2.

The Do Little strain exhibited high degrees of resistance to toxaphene, lindane and dieldrin whilst no resistance was shown to arsenic, DDT, carbaryl or the organophosphorus compounds. The Amatikulu strain showed a moderate level of resistance to carbophenothion, dicrotophos, dioxathion and quintiofos and a high degree of resistance to arsenic, carbaryl, benoxophos and diazinon. The Ongoye 716 strain exhibited a moderate level of resistance to arsenic, DDT, dicrotophos, dioxathion, ethion and fenithrothion and a high degree of resistance to toxaphene, dieldrin, benoxophos, carbophenothion, diazinon and quintiofos. Low levels of resistance, < 3 ×, were shown to chlorfenvinphos and coumaphos. No resistance was shown to bromophos ethyl and chlorpyrifos. Bromophos ethyl was more effective against the organophosphorus resistant Amatikulu strain than against susceptible strains. This negative correlation has been discussed by Shaw, Cook and Carson[5].

Engorged females

A comparison of the logarithm dose/probit mortality regression lines obtained using dioxathion, bromophos ethyl and the diamidine ixodicide, amitraz, for the Kwanyanga and Amatikulu strains is illustrated in Fig. 4.

Engorged females of the Amatikulu strain resisted higher levels of dioxathion than did those of the Kwanyanga strain. Bromophos ethyl was more effective against the organophosphorus resistant Amatikulu strain than against the Kwanyanga strain. No difference was shown in the response of these strains to treatment with amitraz.

Hand-Spraying Trial

A greater number of engorged female ticks of the Amatikulu strain laid viable egg batches after treatment with dioxathion than did those of the Kwanyanga strain. The greater number of engorged female ticks of the Amatikulu strain collected 8, 9 and 10 d after treatment with dioxathion showed that more nymphal stages of this tick strain had survived treatment than had those of the Kwanyanga strain. No difference was observed in the response of these two tick strains to treatment with amitraz (Fig. 5).

Table 2: A COMPARISON OF THE SUSCEPTIBILITY OF LAVAE OF THE KWANYANGA, DO LITTLE, AMATIKULU AND ONGOYE 716 STRAINS OF *BOOPHILUS MICROPLUS*

Ixodicide	Kwanyange LC 99 (%)	Do Little LC 99 (%)	FOR	Amatikulu LC 99 (%)	FOR	Ongoye 716 LC 99 (%)	FOR
Arsenic	2,3 1,0/2,8	1,9 1,6/2,4	—1,2	9,9 3,4/>10	4,3	5,2 2,7/>10	2,2
DDT	0,083 –	0,081 0,025/>10	–1,02	0,094 0,065/0,15	1,1	0,31 0,24/0,43	3,7
Dieldrin	0,058 0,015/1,4	>10 6,7/>10	>172	0,15 0,047/1,3	2,6	9,3 1,98/>10	160
Lindane	0,16 0,011/>10	>10 3,6/>10	>63	0,026 0,0017/>10	–6,2	0,038 0,0065/>10	–4,2
Toxaphene	0,17 0,11/0,3	>10 1,3/>10	>59	0,25 0,16/0,46	1,5	6,0 3,4/>10	35
Carbaryl	0,0075	0,0066	–1,1	0,69	92	*	*
Benoxophos	*0,0062* –	*0,0013* –	–4,8	*0,38* –	*61*	0,39 –	63
Bromophos Ethyl	0,052 –	0,0071 0,0056/0,0097	–7,3	0,0011 –	–47	0,072 –	1,4
Carbophenothion	0,0073 0,0021/1,8	0,0016 0,00072/0,051	–4,6	0,044 –	6	0,15 0,075/>10	21
Chlorpyrifos	0,00046 0,00036/0,00063	0,00032 –	–1,4	*0,00017* –	–2,7	*0,00046* 0,00026/0,012	*1*
Chlorfenvinphos	0,0025 0,0025/0,015	0,0013 0,00078/0,027	–1,9	0,0045 0,0026/0,053	1,8	0,0036 0,003/0,005	1,4
Coumaphos	0,0083 0,0032/0,11	0,0026 0,0013/0,037	–3,2	0,0073 0,0063/0,0088	–1,1	*	*
Díazinon	*0,00029* 0,00026/0,00033	*0,00091* 0,00076/0,0012	*3,1*	*0,059* –	*203*	*0,21* 0,042/>10	*724*
Dicrotophos	0,037 0,033/0,043	0,092	2,5	0,17 –	4,6	0,2 0,17/0,27	5,4
Dioxathion	0,00087 0,00057/0,0027	0,0004 0,00036/0,00047	2,2	0,0063 0,0043/0,013	7,2	0,01 0,0068/0,026	12
Ethion	0,00057 0,0005/0,00069	0,0007 –	1,2	0,0019 0,0015/0,0025	3,3	0,0057 0,0036/0,015	10
Fenitrothion	0,0018 –	0,0072 0,0039/0,029	4	0,0012 –	–1,5	0,0086 –	4,7
Quintiofos	0,0013 0,0011/0,0016	0,001 0,00046/0,32	–1,3	0,0072 0,0044/0,024	5,5	0,078 0,017/>10	60

Where available, the 95 % fiducial limits obtained appear directly below the LC 99 (%) figures.
FOR = Factor of resistance determined by comparison with the Kwanyanga Strain.
– = Confidence limits not calculated, data heterogenous
* = Not tested

DISCUSSION

Four basic types of resistance, arsenic, organochlorine, DDT and organophosphorus/carbamate, are shown for *B. microplus* and as such, constitute the first records of resistance to ixodicides by this tick in Africa.

The results suggest that the trends of the organophosphorus resistance shown are, apart from the reduced susceptibility of the Ongoye 716 to ethion, similar to those in *B. microplus* in Australia and in general may be considered to be at the stage of that of the Ridgelands strain. Shaw *et al*[5] have indicated that the negative correlation factor obtained with bromophos ethyl in the testing of *Boophilus* larvae characterises strains so responding as being of this type, but not of the more advanced, Biarra type.

Cross resistance within both the organochlorine and organophosphorus groups of ixodicides is shown. Neither the organochlorine compound, dieldrin, nor the organophosphorus compound, ethion, have been used as ixodicides in southern Africa but high levels of resistance are revealed for dieldrin in the toxaphene resistant Do Little and Ongoye 716 strains and a significant level of resistance for ethion is shown by the dioxathion resistant Ongoye 716 strain.

A continuing role for amitraz, bromophos ethyl and chlorfenvinphos in the control of the more resistant strains of these ticks is indicated by the results.

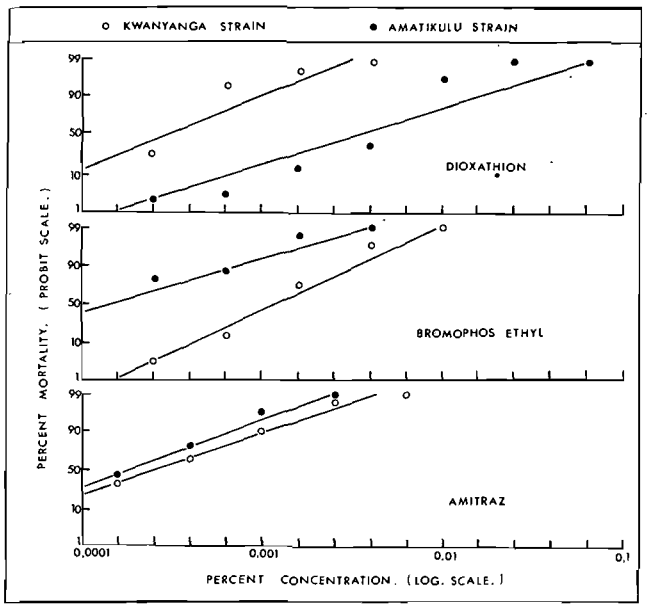

Fig. 4. A comparison of the effect of a range of test concentrations of three different ixodicides on engorged females of two strains of *Boophilus microplus*.

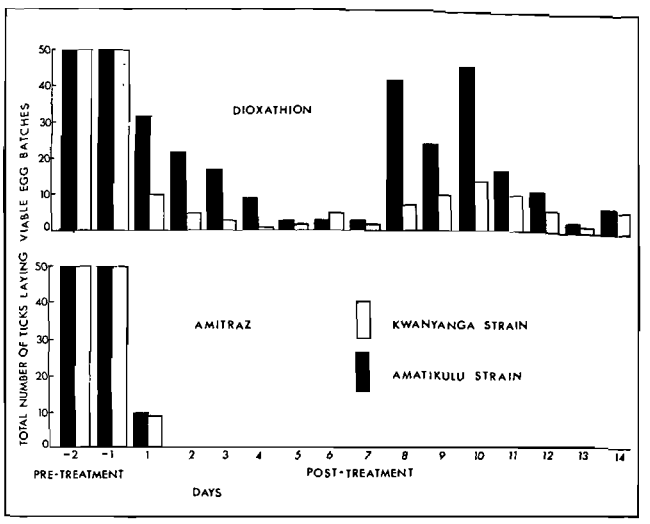

Fig. 5. *A comparison of the effect of two different ixodicides on the viability of egg batches laid by engorged females of two strains of Boophilus microplus.*

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HUMAN BABESIOSIS

D.W. BROCKLESBY*

INTRODUCTION

It is surely only a matter of time before the first human case of infection with *Babesia* is described from the Continent of Africa. One assumes this against a background of descriptions from Yugoslavia, Ireland, Eastern and Western U.S.A., Mexico, France, U.S.S.R. and Scotland, and it is easy to presume that infection must be worldwide. It is interesting to note, however, that all clinical cases have been reported from temperate regions, where blood smears are not routinely examined, and that, so far, only two species of *Babesia* have been implicated, *B. microti* and *B. divergens*.

HISTORICAL

Whilst giving full credit to Škrabalo & Deanović[33 34] and Škrabalo et al[35] for publishing the first convincing description of such a case, we should not forget that earlier authors had at least considered the possibility. As early as 1904 Lingard and Jennings[19] wrote a paper entitled "A preliminary note on a pyroplasmosis found in man and in some of the lower animals". In the same year Wilson and Chowning[39] published a paper in the Journal of Infectious Diseases under the intriguing title "Studies in Pyroplasmosis Hominis ('Spotted Fever' or 'Tick Fever' of the Rocky Mountains)". This is a long and detailed description of work that led the authors to believe that the cause of the disease was a large *Babesia* species "closely related to *Pyroplasma bigeminum, Pyroplasma canis, Pyroplasma ovis* and *Pyroplasma equi* (sic)". The parasite, which was found in a high proportion of cases, was named *Pyroplasma hominis:* the paper is illustrated with paintings and drawings which are completely convincing. Another interesting aspect of their work is that they apparently succeeded in infecting rabbits by blood inoculation from sick patients.

In 1920 Wright[40] published "A Preliminary Paper on observations on Blackwater Fever (Haemoglobinuria) in the Coorg Province 1917–1918. Especially as regards its Etiological Factor, namely, a Protozoan Parasite of the Genus Piroplasma in Conjunction with the Malarial Plasmodium; or a New and Undescribed Species of *Laverania Malariae* (Donovan's theory)". In his Research Memoir on Blackwater Fever in Southern Rhodesia, Thomson[36] mentions that the similarity between blackwater fever in man and redwater in cattle had led to a belief that an organism related to the Babesidae might be responsible for the production of blackwater fever; he refers to Wright's illustrations but says they were simply forms of *Plasmodium falciparum.* Thomson frequently encountered pear-shaped malignant tertian parasites in patients in Rhodesia but these, he says, were easily proved to be merely morphological variations of the asexual forms of *P. falciparum:* he concluded that there was no evidence that man is ever infected with *Babesia.*

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Babesia parasites have, however, been detected in the blood of some higher primates. The first mention of such organisms was that of Ross[27] who described *Piroplasma pitheci* in a *Cercopithecus* monkey in Uganda: this was a typical piroplasma of the *B. canis* or *B. bigemina* type. Similar parasites are encountered from time to time, normally after removal of the spleen, and Garnham[8] suggested that the organisms may be *B. bigemina,* or possibly an allied species, which accidentally infect monkeys; morphological differences could be due to a change of host.

THE MODERN LITERATURE

The occurrence of human piroplasm infections has excited a wide interest and accounts of some of the recorded cases will, therefore, be given in some detail.

The first Yugoslav case

In June 1956 a 33 year-old tailor was admitted to the Department of Medicine of the University of Zagreb in a grave condition. He had been splenectomised 11 years previously as a result of a serious traffic accident; he drank about one litre of spirits a day and one wonders whether this might have contributed to his susceptibility. On admission to hospital the patient exhibited the following signs; chocolate-brown colour of the skin, fever, anaemia and haemoglobinuria. He had been taken ill eight days before but had only consulted a doctor the day before he was sent to hospital. Haematological investigations revealed an erythrocyte count between 1,0 and 1,32 $\times 10^6$ and severe haemolysis; the patient was treated with penicillin, streptomycin, cortisone, infusion of 5 % glucose and saline with vitamin C and B, and intravenous novocaine. However, he lost consciousness during the second day and died early on the third day after admission.

Smears of peripheral blood and sternal marrow were made on the last day. Many intra-erythrocytic parasites were found and, after consultation with specialists, the authors concluded that it was a case of piroplasmosis; the species of piroplasm was difficult to determine but it was thought likely to be *B. bovis.*

The man had lived in a village called Strmec about 10 km from Zagreb and the authors visited the area; they established that the patient owned cattle which he grazed on pastures along the river Sava. He tended the cattle himself and there were plenty of ticks on the pastures *(Dermacentor silvarum* and *Ixodes ricinus).* Only 1 km away there had been cases of redwater in local cattle two weeks before the patient first became ill.

The Californian case

This case was first mentioned by Braff and Condit[4] and was fully described by Scholtens et al[30]. The person involved was a 46 year-old resident of San Francisco who was an amateur photographer whose interest in

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nature took him on field trips into relatively isolated coastal areas. He had been splenectomised two years previously because of an hereditary spherocytosis. An illness manifested by chills and anaemia was diagnosed as malaria; chloroquine therapy was followed by recovery. Weekly administration of chloroquine (250 mg weekly) was continued for 3½ months.

Blood films taken during the illness revealed non-pigmented intra-erythrocytic parasites in at least three distinct morphological forms; small ring forms resembling malaria parasites, older amoeboid forms, semi-mature forms consisting of four distinct elements in various stages of development up to mature Maltese cross tetrads. Blood films taken later were all negative and attempts to infect a group of six mice and one burro were unsuccessful. Serum samples were taken two months after the illness and sent to various laboratories. Indirect fluorescent antibody (IFA) tests against 10 *Plasmodium* species were negative. Agar-gel precipitin tests, and IFA tests against *B. caballi* and *B. equi* were also negative; the complement fixation test, however, was positive at the 1:5 dilution. A tube latex-agglutination test (TLA) using a *B. canis* soluble serum antigen was positive at 1:80 (four samples from apparently normal human beings were negative at 1:2). A further TLA test was carried out on a serum sample obtained from the patient two months later and was positive at 1:640.

The results of this experiment were somewhat inconclusive. It seems most likely, however, that the parasite involved had originated in a wild rodent and it could well have been *B. microti*

The Irish case

The third case was described in two publications by Fitzpatrick et al[7]. The patient was a 47 year-old fisherman who had a posterior gastroenterostomy and vagotomy during which, because of technical difficulties, the spleen was torn and had to be removed. Three months later, in mid-August, he went on a caravan holiday in County Galway, Eire, and it seems certain that this is where he became infected; three cases of bovine redwater were reported from that area in the same month. The man became ill on 29th August and noticed that he was passing blood in his urine. The next day he became jaundiced and on 31st August his doctor sent him into hospital. Tests were carried out and a clinical diagnosis of Weil's disease was made. On 2nd September he was transferred to the renal unit of Belfast City Hospital; by this time he was anuric, moribund, disoriented and not able to give a history. The jaundice had progressed and was a greenish-bronze colour. He was treated with massive doses of penicillin, hydrocortisone and peritoneal dialysis but continued to deteriorate; by the 4th September the diagnosis had veered towards serum hepatitis. The patient was severely anaemic and the red cells were macrocytic, with anisocytosis, polychromasia and many distorted and fragmented forms.

"A striking feature was the presence of numerous intra-erythrocytic inclusions".

The above is a very brief account of the extensive investigations made by the doctors who so strenuously struggled to save the patient's life. Unfortunately he died on 5th September just as an exchange transfusion was in preparation.

The laboratory diagnosis at this stage was blackwater fever following a malignant tertian infection, probably transmitted by a blood transfusion. This opinion was held for several weeks but continuing investigations made it increasingly unlikely. Finally it was reported by the Malaria Reference Laboratory in England that the infection was piroplasmosis caused by *Babesia divergens*.

The first Massachusetts case

This, the fourth reported case of human babesiosis, has excited widespread interest and research for two reasons: firstly, the patient had not been splenectomised and secondly, the causal parasite was isolated in experimental animals and has been characterised as *Babesia microti*.

The case was first reported by Benson et al[3] and a full case report was given later by Western et al[38]. The patient involved was a 59 year-old widow who was admitted to a New Jersey hospital after two weeks illness during which she suffered from fever, headache and crampy abdominal pain. Blood films showed numerous intra-erythrocytic rings like *P. falciparum* trophozoites. She had been taken ill whilst on holiday on Nantucket Island, off the Massachusetts coast, and she recalled having removed a deeply embedded tick from her own suprasternal notch a few weeks earlier; she had also regularly removed ticks from her dachshund. Chloroquine was given orally for 30 days and parasitised red cells gradually decreased in number until the 18th day when none could be found. The patient returned to Nantucket Island and took chloroquine twice a week for a further eight weeks. Weekly blood films were taken and these were positive with very rare parasites being found on two occasions. However, the patient remained well and no further treatment was given.

A close examination of the parasites seen in blood films led to a diagnosis of Babesiosis rather than malaria; this was based on the presence of "tetrad" forms (= Maltese crosses) and the absence of malarial pigment. IFA tests against the four *Plasmodium* species that infect humans were carried out in two different laboratories; one of these reported positive reactions that were stronger against *P. falciparum* than the other species.

Six days after she was admitted to hospital the patient was bled and a variety of laboratory animals was inoculated with her blood; the parasite was established in a hamster and in a splenectomised rhesus monkey. Details of the isolation of this parasite,which has become known as the "Gray" strain of *Babesia microti*, have been given by Gleason et al[12]. The patient's blood was collected into two anti-coagulants, sodium oxalate and EDTA, and samples of each were inoculated intraperitoneally into the following intact animals: two CFW mice, one Sherman strain rat and a golden hamster. Additionally, a dog was injected intravenously and intraperitoneally with a sample of the oxalated blood. The parasite only became established in the hamster that received the EDTA blood and all subsequent animal passages were derived from this hamster. From the passage hamster blood was inoculated into two gerbils which became infected; the pattern of infection was like that seen in the hamsters.

Van Peenen & Healy[37] showed that the prairie vole *(Microtus ochrogaster)* was susceptible to infection. Ristic et al[26] established the parasite in splenectomised

rhesus monkeys and were able to infect hamsters from these monkeys, but not dogs.

The second Yugoslav case

This case which was briefly described by Škrabalo (as a personal communication from Harambašić, 1969)[32] involved a 27 year-old male factory worker who had been splenectomised three years previously. He was brought to the hospital of the University of Zagreb Medical School with symptoms of extreme prostration, chills, fever, jaundice, diarrhoea, vomiting and frequent passage of dark red urine; these symptoms had an acute onset on the day of admission to hospital. Laboratory investigation showed a severe progressive anaemia, leucocytosis with immature neutrophils, lymphopenia and later monocytosis, hyperbilirubinaemia and a slight increase in gamma globulins. Three days after admission *B. divergens* was discovered in blood films; treatment with diminazene aceturate ("Berenil") was given but the exact dosage is not mentioned (3 mℓ intramuscularly). The patient's condition did not improve since marked anuria, uraemia and extreme anaemia had already developed in spite of intravenous fluids and transfusions of washed red cells. The patient died five days after admission, with haemorrhagic pulmonary oedema.

The second Massachusetts case

In 1974 Anderson, Cassady & Healy[1] reported details of the sixth documented case of human piroplasmosis. The infection was acquired on Nantucket Island, close to the area of the first Massachusetts case, by a 48 year-old woman who had not been splenectomised. She was admitted to hospital complaining of chills, fever and aching of the legs; the fever, which was relieved by aspirin, occurred about once a day and was often accompanied by an uncontrollable urge to cry. *Babesia* (5/1000 RBC) were identified in blood smears two days after admission to hospital and chloroquine therapy was immediately initiated. The response was dramatic; the patient began to feel better and the anaemia was controlled with blood transfusions. The initial dose of chloroquine was 1,5 G and the patient was maintained on 0,5 G per day for the following month and then twice a week for the next six months. Febrile episodes occurred during the first eight days of chloroquine therapy and the parasitaemia declined to 1/1000 RBC by the second day after treatment was begun. When the patient was discharged after three weeks in hospital no parasites could be detected.

The parasite was isolated in gerbils, the only suitable animal available at the local pet shop, and later in hamsters, at the Centre for Disease Control in Atlanta. Immunofluorescent tests confirmed that the parasite was *Babesia microti*.

The Mexican experience (Latent infection in three people)

Strikingly interesting results were reported by Osorno[22]. He carried out indirect haemagglutination (IHA) tests,using *B. canis* antigen, on 101 serum samples from human beings living along the Gulf Coast in Mexico. Thirty-eight of these reacted at titres between 1:10 and 1:80. He collected individual blood samples from these thirty-eight people and inoculated splenectomised hamsters. Hamsters inoculated with blood from three of the individuals showed babesia in their peripheral blood; and the parasite was established by subpassage into additional hamsters (Osorno et al[23]). This work described the latent form of human babesiosis for the first time and provided the first intimation that subclinical infection in man could be common

The two French cases

Gorenflot, Piette & Marchand[13] describe a case of infection in a 53 year-old splenectomised man and include some scanning electron micrographs of infected human erythocytes. They felt that *B. divergens* was the likely culprit but were not able to attempt isolation in cattle. The second case, which was also not fatal, occurred in a 61 year-old splenectomised female who had recently been camping in the valley of the River Loire[2].

The five cases of Nantucket Island

This most interesting work is described in two papers[15] [28]; the first[15] is a brief account of the remarkable results. One is lulled by the title "Human Babesiosis – Reservoir of infection on Nantucket Island" into thinking that this must be a rather dull paper concerning the piroplasms of the local rodents. Indeed this is partially true and the authors found that the local field mice and deer mice were highly infected with *Babesia* species. However, they also made many visits to local physicians and asked that the blood of patients showing chills and fever, lethargy, myalgia and headache, should be examined microscopically. They continue as follows: "During July and August 1975, five patients with this spectrum of symptoms proved to have circulating parasites that were consistent in appearance with *B. microti.*"

In each case successful subinoculation into hamsters was made. Two further cases came to light later the same year and both were people who had been ill for some time. One was a summer resident of a nearby island called Martha's Vineyard and was taken ill in Washington, and the second was a Nantucket Island resident. The second paper[28] is an excellent account of the clinical features of this "new" disease. None of these seven people was splenectomised and neither was an additional symptomless carrier in Georgia[16]. So these three papers added seven clinical cases and one latent case to the growing list of human *Babesia* infections.

The Russian case

Rabinovich et al[25] gave a brief report concerning a fatal case, diagnosed after death, in a 49 year-old female from a rural area of Georgia. The causal parasite was said to be *"Babesia caucasica* species close to *B. divergens"*.

The Scottish case (Entrican et al, 1979)[6]

In August 1977 a 34 year-old man, suffering from Hodgkin's disease since 1974, became severely ill and was admitted to hospital in Inverness, Scotland. As part of his earlier treatment he had been splenectomised and subjected to irradiation. On admission he

was feverish (37,5°C) and was suffering from extreme tiredness, vomiting, diarrhoea and was passing diminishing volumes of dark red urine. His skin was dusky yellow, conjunctivae were suffused and mucosae were jaundiced. There was such an intense haemolysis that it was not possible to read the erythrocyte sedimentation rate. Blood smears were examined and revealed a very heavy infection of erythrocytes (up to 70 or 80 per cent of the cells) with an intracellular parasite; up to eight parasites were seen in a single cell. Babesiosis was suspected by the Haematologist and arrangements were made to obtain veterinary babesicidal drugs. In the meantime the patient was treated with intravenous quinine and later with chloroquine and pyrimethamine; he was also transfused with concentrated red blood cells. During the next three days the parasitaemia fell to 14 per cent but renal failure became progressive and, with the development of severe chest pains, anaemic pericarditis was suspected. In spite of two haemodialyses a pericardial rub became obvious and there were signs of pericardial effusion; 500 mℓ of blood-stained fluid were removed but the patient's condition rapidly worsened and he died.

Strenuous efforts were made to isolate and identify the *Babesia* species involved and these proved to be most interesting. Blood from the patient, taken at a time when the parasitaemia was 50 per cent, was inoculated into rats, cotton rats, guinea pigs, mice, nude mice, hamsters and gerbils. Only the gerbils became infected; the parasite multiplied well and was easy to maintain in these animals and parasitaemias of about 60 per cent were commonplace. Blood from the patient was also sent to the Institute for Research on Animal Diseases at Compton where 4 mℓ was inoculated intravenously and subcutaneously into a six month-old splenectomised calf. Four days later parasites appeared in the red cells of the calf and the parasitaemia reached a peak of 9/1000 R.B.C. on Day seven. The parasites appeared to be identical with *Babesia divergens*. The calf was later challenged with a laboratory strain of *B. divergens* and was solidly immune. Subsequent serological tests (IFA) were carried out at the Central Veterinary Laboratory, Weybridge: these compared the antigenic characteristics of the parasite from the patient's blood, and from infected gerbils, with *B. divergens, B. major* and *B. microti* and confirmed that the parasite was *B. divergens*. It was somewhat surprising to find that the parasite isolated from this Scottish case grew with equal facility in gerbils and cattle; the obvious next step was to see whether bovine strains of *B. divergens* would prove to be capable of growing in gerbils and this indeed was shown by Lewis and Williams[18] to be the case. High parasitaemias occurred in splenectomised and intact gerbils and the parasite could readily be transferred back to cattle. This is the first time that a bovine *Babesia* species has been transmitted to and maintained in a laboratory animal and it could well be developed as a useful laboratory model system. It will also be interesting to see whether the gerbil is susceptible to other "unlikely" parasites.

Other American cases

A number of further cases have been described from the U.S.A. Grundwaldt[14] gave an account of three cases in an article entitled "Babesiosis on Shelter Island". All these were apparently due to infection with *B. microti* and one of them is of some interest as it occurred in a man who had undergone splenectomy a year before during surgery for hiatus hernia. Another case, from Long Island, was described by Parry et al[24] and, in connection with the failure of chloroquine as a chemotherapeutic agent, Miller, Neva & Gill[20] described an infection in a man who had just returned from a holiday on Martha's Vineyard. In 1979, Ruebush et al[29] published a paper describing the 15th American case; this occurred in a 65 year-old man, a resident of Nantucket Island, and failed to respond to chloroquine therapy. The doctors then tried diminazene and this had a dramatic effect on the parasites which quickly became impossible to detect in blood smears; subinoculations into hamsters, however, showed that they were still present eight days after withdrawal of the drug but that they had apparently been eliminated by the 39th day. The patient was discharged from hospital but a few days later he had to be re-admitted because of acute neurological complications (Landry-Guillain-Barré Syndrome). The authors felt that diminazene was probably the cause of the neuropathy.

Some related observations

The cases of human piroplasmosis described above have naturally resulted in a good deal of further investigation and speculation. The first to move into action, stimulated by an examination of blood films taken from the first case, were Garnham & Bray[9] who decided it would be interesting to observe the reaction of splenectomised chimpanzees to inoculation with *Babesia* species. Bovine blood infected with *B. divergens* was taken from the Central Veterinary Laboratory in England to the Institute of Medical Research in Liberia where it was inoculated into two chimpanzees, one of which was spelenctomised at the same time; the other had been splenectomised three years earlier. Both animals became infected with parasites being found on the sixth day in the old splenectomised animal but not until the 26th day in the second chimpanzee. The latter animal became very ill, with haemoglobininaemia and prostration, but made a surprising recovery; parasites gradually disappeared and after a month the chimpanzee's blood was inoculated into a splenectomised calf with negative results. Garnham & Voller[11] carried out experiments with rhesus monkeys and achieved similar results; the monkeys developed a higher parasitaemia than the chimpanzee. Antibodies (IFA test) were found in the splenectomised rhesus monkeys but did not develop in an intact monkey that was injected with heavy inocula of *B. divergens*. They concluded that the IFA test was therefore not likely to be of much use for the detection of asymptomatic human carriers.

The Irish case (Fitzpatrick et al, 1969[7]) was also followed up by Garnham et al[10]. They visited the site in Galway where the unfortunate Irishman had contracted the disease twelve months earlier. Their main aim was to collect blood from local agricultural workers and inoculate this into splenectomised calves. Unfortunately they were diverted from this admirable intention by the appearance of a detachment of the Irish army who were acting as film extras during the shooting of the MGM production "Alfred the Great". They took blood from 35 soldiers and this was injected into calves at the Weybridge laboratory but with negative results.

This was not surprising since the troops had only been in the area for about two weeks and so had not had much opportunity to become infected; it was also evident, from their failure to transmit *Babesia* with ticks collected from the vegetation, that the infection rate in the local ticks was very low.

Shortt & Blackie[31] also demonstrated the susceptibility of a splenectomised primate to infection with piroplasms. They isolated a strain of *B. microti* from a mole *(Talpa europoea)* and established it in multimammate rats *(Mastomys coucha)*. A seven-day-old infection of the rats was used to inoculate a splenectomised rhesus monkey intravenously and intraperitoneally: *Babesia* parasites appeared three weeks later and increased to produce a heavy parasitaemia. The monkey exhibited a slight anaemia but was not otherwise much affected.

DISCUSSION AND CONCLUSIONS

The recorded cases of human piroplasmosis could well represent the tip of an iceberg. People in rural tick infested areas throughout the world are constantly exposed to attack by ticks and latent infections could be common. Garnham & Bray[9] even suggested that latent babesiosis may have unknown pathogenic effects and went on to say: ". . . for instance, multiple sclerosis particularly affects farm workers; could the piroplasm be an etiologic factor of this disease?"

This idea may seem a little far fetched but should nevertheless be considered; perhaps it would be worthwhile to collect blood from MS sufferers and inject it into splenectomised domestic and laboratory animals.

It is hazardous to attempt to draw any general conclusions from the cases so far recorded but it does seem clear that they fall into two groups. Apart from the recently reported cases from France, the European cases were all fatal, they all occurred in splenectomised hosts and they were all due to infection with bovine *Babesia* species. The American patients, with one exception, were not splenectomised and all were infected with the rodent parasite, *B. microti.* Early faith in chloroquine therapy has been supplanted by a feeling that *B. microti* infections in intact humans are self-limiting and that the anti-malarial drug has no effect. So we have a severe life-threatening form of the disease in Europe and a less dramatic form in America. Treatment should be conservative in the latter but affected patients with the European form of the disease may have to be treated with one of the veterinary babesicides. But which drug should be chosen? In 1976 I posed the question, supposing that a specialist in piroplasms became accidentally infected and that his physician asked for help. At that time I came down in favour of diminazene since it was the only veterinary babesicide that had been used in man. Nash[21], in the annual Report of the West African Institute for Trypanosomiasis Research for 1957, reported that intramuscular injections of 2 mg/kg were well tolerated. Hutchinson & Watson[17] treated 17 patients by giving seven consecutive daily deep intramuscular injections in the form of a 2 % solution in 5 % dextrose at a daily dosage of 2 mg/kg; there were no untoward effects apart from a persistent but harmless albuminuria in some patients. But we now have the report by Ruebush et al[29] that suggests that, diminazene may not be so good after all; it had a dramatic clinical effect but the patient later suffered from an acute polyneuritis. In addition, the intramuscular injections were said to be extremely painful. Nevertheless, diminazene remains the only known effective drug with a record of use in man and one is forced to the conclusion that it should be used in a life-threatening situation. One hopes that other drugs, such as imidocarb, will soon be evaluated for toxicity in experimental primates.

Further surveys could, or should, be carried out and the use of the gerbil as a recipient should make this an easier matter. The recent work of Chisholm et al[5] shows that the IFA test would be a useful survey tool for *B. microti* infections.

Human babesiosis can no longer be regarded as a medical oddity.

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SOME ASPECTS OF THE EPIDEMIOLOGY OF EQUINE BABESIOSIS

A. LITTLEJOHN and EILEEN M. WALKER

ABSTRACT: Littlejohn A.; Walker, Eileen M. **Some aspects of the epidemiology of equine babesiosis.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 308-310 (En). Equine Physiology Research Division, Faculty of Veterinary Science, Onderstepoort, University of Pretoria, Republic of South Africa.

The sex, coat colour, age, province and month of occurrence of every case of babesiosis was recorded in a horse population of 5856 in South Africa and Rhodesia. A total of 115 cases were recorded during the period 1973-01-01 to 1973-12-31. Chi-squared tests were used to evaluate the significance of frequencies. Significant biases in the distribution of cases of babesiosis were found with regard to season ($P < 0,05$), sex ($P < 0,001$) and coat colour ($P < 0,01$).

INTRODUCTION

Despite the fact that equine babesiosis is known to be widespread in Southern Africa, there is little information about the epidemiology of the disease. Different species of equidae vary in susceptibility to the disease[9] and entire males were considered by Littlejohn[4] to be less resistant than mares. However, there are no published statistics about the incidence of the disease in Southern Africa, nor in different provinces of the Republic of South Africa. The lack of such information makes it difficult to assess the economic significance of equine babesiosis and hinders rational investigation of its effects upon the host.

The present study forms part of a survey of equine diseases in Southern Africa[5] and it investigates several basic aspects of the epidemiology of equine babesiosis, namely:

a. The overall incidence in South Africa and Rhodesia.
b. The incidence in three provinces of the Republic of South Africa
d. Seasonal effects on the incidence of equine babesiosis.
d. The influence of coat colour, sex and age.

MATERIALS AND METHODS

Regular monthly returns were provided by twelve equine practitioners in the Cape Province, Natal, Transvaal and Rhodesia. Figures from the Orange Free State were not available. The total number, sex and the coat colour of all the horses in each practice were recorded. The sex, coat colour and age of every case of babesiosis attended by the twelve practitioners were recorded, in addition to the province and month in which it occurred. Saddle horses only were included in the survey; ponies and draught horses were excluded, as were mules and donkeys. Breeds were not specified, but the location and composition of the twelve practices indicated that the vast majority of the population surveyed were Thoroughbreds.

The figures provided in the monthly returns were checked, punch-carded and stored in the IBM 360/50 Computer of the University of Pretoria Computer Centre, from which information was extracted as required. However, since some of the analyses were highly significant from a statistical point of view, the figures for babesiosis were rechecked by visual inspection of each monthly return from each veterinarian. The pooled data was then analysed by simple arithmetic using chi-squared tests for determinations of significance.

RESULTS

Population sample

The survey covered a saddle horse population of 5856. Of this number, 3567 were female and 2289 were male. Geographical locations were as follows: Cape Province, 1626; Natal, 1080; Transvaal, 550; Rhodesia, 2600. The coat colour distribution of the population sample was as follows: Black, 2,35 %; brown 20,0 %; chestnut, 24,85 %; grey, 6,0 %; roan, 0,41 %; bay, 45,43 %; skewbald/piebald, 0,37 %; dun, 0,58 %.

Incidence

A total of 115 cases of babesiosis was recorded from 1st January to 31st December 1973. The incidence for that year in the population surveyed was therefore 1,88 %. The incidence in the Cape Province was considerably less than that of Natal, Transvaal or Rhodesia (Fig. 1) but the difference was not statistically significant.

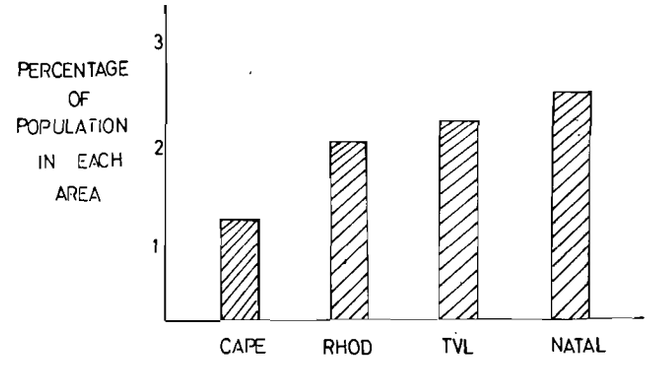

Fig. 1. The incidence of equine babesiosis in Rhodesia and South Africa in 1973.

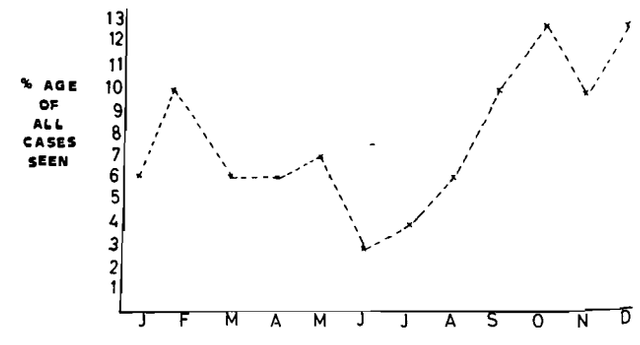

Fig. 2. The monthly distribution of equine babesiosis cases in Rhodesia and South Africa during 1973.

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Seasonal distribution

The monthly distribution of cases is illustrated in Fig. 2. There was a tendency for the incidence of the condition to increase during the summer months but this increase was not statistically significant. However, when data from the summer rainfall areas (Natal, Transvaal and Rhodesia) was analysed, the tendency was of probable significance ($P < 0,05$).

Sex distribution

There was a significant bias in the sex distribution of equine babesiosis (Fig. 3). With chi-squared = 45,5 ($P < 0,001$), the null hypothesis that each sex was affected in proportion to its percentage of the population sample was rejected and a significant bias towards males as regards overall incidence was therefore accepted.

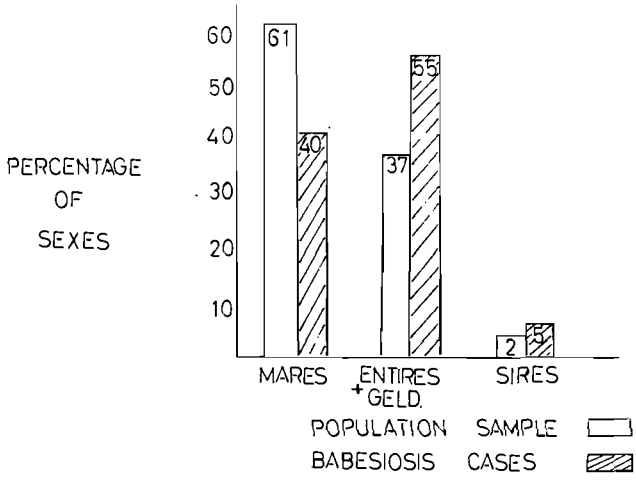

Fig. 3. Sex distribution of cases of equine babesiosis.

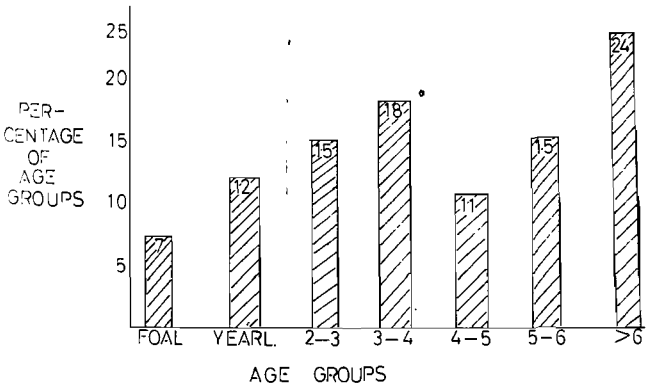

Fig. 4. Age distribution of equine babesiosis cases in Rhodesia and South Africa in 1973.

Age distribution

Since the exact ages of the population sample were not known, the possible significance of age-group distributions could not be evaluated. However, it was evident that cases of babesiosis were seen most frequently in yearlings and in two- and three year olds (Fig. 4).

Coat colour

The coat colour probably has a significant effect on the incidence of colour categories of horses to babesiosis (chi-squared = 14,3 $P < 0,05$). However, when the frequency of chestnut was compared with that of other colours, the value of Chi-squared was significant at $P < 0,01$ (Fig. 5).

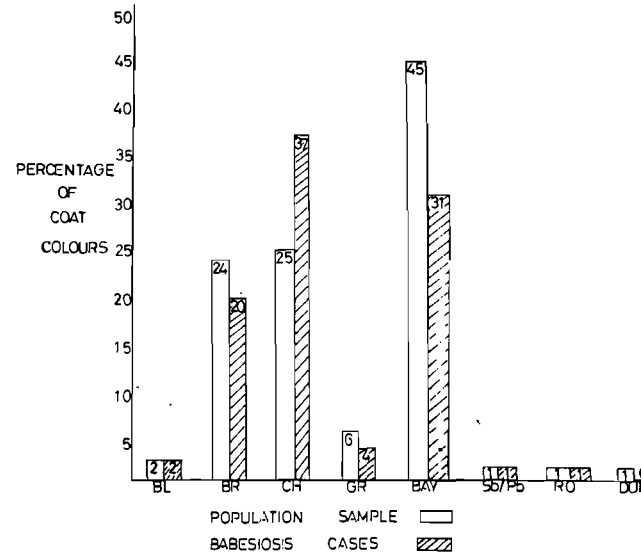

Fig. 5. Coat colour distribution of equine babesiosis cases in Rhodesia and South Africa in 1973.

DISCUSSION

Overall incidence

A frequency of 188 cases of babesiosis per annum per 10 000 saddle horses indicates that it is at present the most serious infectious disease of horses in Southern Africa. In the survey of Littlejohn *et al*[5], the percentage cases of babesiosis treated exceeded the percentages of every other infectious disease of horses including infectious respiratory disease and African horsesickness. During the year 1973 only six cases of Horsesickness were recorded among the same population of 5856 – a figure which reflects the efficient control over the disease achieved by the widespread use of the multivalent vaccine produced at Onderstepoort.

Seasonal distribution

A probably significant bias towards the summer months in the summer rainfall areas is almost certainly associated with the biology of the vectors concerned. Tick populations increase dramatically during the summer months in the Transvaal[6].

Despite the higher incidence during summer months, however, babesiosis remains a threat throughout the year in Southern Africa and control measures should not be relaxed during winter months.

Sex distribution

The highly significant bias towards male horses cannot be satisfactorily explained. The higher than expected frequency among sires (six cases occurred whereas only one case was the expected frequency) can partly be

explained by the high percentage of imported stock in this sex category. In South Africa 77 % of Thoroughbred sires at stud are imported, whereas only 8 % of mares are imported (Yearling Sale Catalogue, Witwatersrand Agricultural Society, 1974). Thus the female population as a whole might be expected to possess a greater level of premunity than the sire population. This does not however, apply to the rest of the male population in which both entires and geldings appear to exhibit a significant susceptibility to babesiosis. Conversely it may be that female horses develop greater resistance to babesiosis. If so the mechanism of such resistance should be of considerable interest.

Sex differences in the incidence of haemotropic diseases have so far not been recorded in horses. However, resistance to *B. rodhaini* has been demonstrated in female rodents[2]. The existence of a sex-linked immunogenetic factor cannot be discounted but at this stage further investigation is indicated to exclude other factors which might influence the sex distribution of equine babesiosis.

Age distribution

The possible bias towards yearlings, two- and three-year olds in the distribution of babesiosis in different age groups may be associated with the stresses of breaking, training and racing, plus the movements from stud to sale ring to racing centres and consequent exposures to fresh infections. There may also be factors which induce a high tolerance to babesiosis in aged horses, e.g. a steadily rising antibody titre as horses become older in enzootic areas.

Coat colour

The bias towards chestnut coloured horses in the frequencies of babesiosis cases is rather surprising. Both immunological mechanisms and coat colour in horses are determined by genetic factors, but there is no evidence that the two are linked.

The coat colours of horses are derived from the presence in the coat of two different groups of pigments – black-brown and red-yellow[1]. Basic coat colours are therefore bay, brown, black and chestnut[8]. There is general agreement that chestnut differs from the darker colours by a simple mendelian recessive[13]. The observed and expected frequencies of babesiosis in dark and chestnut coated horses were therefore compared. Roan was included with chestnut and piebald with bay etc because the genes responsible for roan and piebald are epistatic to the basic colours[8]. The results were as follows:

	Chestnut and roan	Bay, black, brown, grey and piebald
Observed frequency	44	71
Expected frequency	30	85

A chi-squared of 8,84 was obtained, a result which is significant at $P < 0,01$.

The presence of specific antibodies in dark-coated horses has not been reported. However, there may be analogies to human malaria. In negroes, resistance to infection with *Plasmodium vivax* is associated with the Duffy negative phonotype FyFy[12] and heterozygosity for the sickle-cell anaemia gene confers resistance to *P. falciparum*. It is possible that resistance to babesiosis in dark-coated horses is associated with different blood group systems.

Sex- and colour-linked associations with diseases in domestic animals are well-known and have been extensively reviewed elsewhere[3 7]. It is possible however, that mechanisms of resistance are associated with resistance to ticks rather than to blood parasites. In cattle, breed, sex and age play significant rôles in resistance to tick infestation[10 11] and these may be important factors in equine babesiosis.

In conclusion, it appears that dark-coated mares have more resistance to babesiosis than chestnut colts. Investigations into the nature of such resistance may prove of value in prophylaxis or treatment of the disease.

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THE DIFFERENTIAL DIAGNOSIS OF THE BOVINE THEILERIAS OF SOUTHERN AFRICA

J.A. LAWRENCE

ABSTRACT: Lawrence J.A.; **The differential diagnosis of the bovine theilerias of southern Africa.** *Journal of the South African Veterinary Association.* (1979) **50** No. 4 311-313 (En). Veterinary Research Laboratory, P.O. Box 8101, Causeway, Zimbabwe Rhodesia.

It is currently thought that the following species or sub-species of *Theileria* occur in cattle in southern Africa: *Theileria parva parva* (East Coast fever), *Theileria parva lawrencei* (Corridor disease), *Theileria parva bovis* (Rhodesian theileriosis), *Theileria mutans* proper (transmitted by *Amblyomma* species), so-called *Theileria mutans* (a non-pathogenic species transmitted by *Rhipicephalus appendiculatus,* possibly identical with *Theileria taurotragi*) and *Theileria velifera.* The parasites can be differentiated on serological, morphological and epidemiological grounds. The four true species are serologically distinct. *T. mutans* proper and *T. velifera* show morphological differences from the other two species in some stages of their development. The *T. parva* group are the only parasites that are commonly pathogenic. Differentiation of the three members of this group is based on differences in the numbers of schizonts and piroplasms present and on differences in the epidemiology of the diseases they cause.

INTRODUCTION

The bovine theilerias of southern Africa have always presented a problem in differential diagnosis, as have those in other parts of the world. The problem has become more complex with the passage of time as the number of species or strains of theileria which is recognised grows. The subject can best be approached by following the history of the development of knowledge of the parasites and describing the diagnostic criteria that have been applied at various times.

HISTORICAL REVIEW

In January, 1902, a new disease appeared in southern Africa, on the commonages of Umtali and Salisbury in what was then known as Southern Rhodesia[14]. The disease was characterized by the presence of large numbers of intraerythrocytic piroplasms and it was at first considered to be a particularly virulent form of babesiosis. It was later identified as East Coast fever; caused by the parasite now known as *Theileria parva,* Theiler, 1904, and it was realised that infection had been introduced with a consignment of cattle imported from Tanganyika late in 1901. The characteristic schizont was described by Koch in 1905 and was considered to be pathognomic of East Coast fever. In 1906 it was recognised that intraerythrocytic piroplasms of a second theileria could be found in cattle and this parasite was named *Theileria mutans,* Theiler, 1906. It could be distinguished in the field from *T. parva* because it was usually non-pathogenic and because it appeared not to produce schizonts.

East Coast fever spread widely and, in its epizootic form, it had a mortality rate of 90 per cent. The effect on the economy of the countries of southern Africa was disastrous and control and eradiction became a major preoccupation of the veterinary authorities. Control measures centred on the detection of infection followed by eradication by quarantine, dipping and sometimes slaughter, and diagnosis was based on the presence of schizonts. Lymph and spleen smears were examined as a routine in any disease investigation and if schizonts were found this constituted an automatic diagnosis of East Coast fever.

It was not until 1928 that it was discovered that this policy was based on a wrong premise. Schizonts were found in a small proportion of calves that were dying from a combination of salmonellosis and bad husbandry[19]. The disease was clearly not East Coast fever and it was realised that the schizonts were those of the parasite known as *T. mutans,* which was present in the calves. A new diagnostic criterion had to be introduced for *T. parva,* namely that in addition to producing schizonts and piroplasms, the parasite caused a disease which had the characteristic clinical, pathological and epidemiological features of East Coast fever.

In 1934 Lawrence[8] encountered a disease on Nuanetsi Ranch in the southern lowveld of Zimbabwe Rhodesia which was quite distinct from East Coast fever but in which schizonts were found consistently in small numbers. Infection was confined to cattle grazing in areas frequented by buffalo and did not persist when the cattle were removed. The disease was subsequently encountered in Zululand and given the name Corridor disease and the parasite was named *Theileria lawrencei,* Neitz, 1955.

In 1936, Lawrence[9] encountered a similar form of theileriosis on Fortuna Farm, in the Eastern Districts of Zimbabwe Rhodesia, which was not associated with buffalo but was distinguishable from East Coast fever in that schizonts were relatively infrequent and were smaller than those of *T. parva* and piroplasms were also infrequent. The disease resembled East Coast fever clinically and pathologically but was not identical and it had a strictly seasonal incidence, coinciding with the peak activity of adult *Rhipicephalus appendiculatus.* It had a lower morbidity and a very much lesser capacity for spread. Neitz[11] named this parasite *Theileria bovis* and, although in a footnote to his paper he declared it to be synonymous with *T. lawrencei,* there is much merit in regarding it as a separate entity. The disease which it causes is probably best named Rhodesian theileriosis, as it appears to be unique to Zimbabwe Rhodesia.

At this stage three disease entities were recognised, distinguishable mainly on epidemiological grounds. There were differences in the clinical and pathological features and, in East Coast fever, schizonts and piroplasms were more numerous than in the other two conditions. *T. mutans* was still generally regarded as a benign parasite, although de Kock et al[4] did incriminate it as the cause of an outbreak of disease at Tzaneen in the Transvaal.

The classification of the theilerias was thrown into confusion by Brocklesby[2] when he demonstrated that, on occasion, *T. lawrencei* would transform on passage

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Table 1: DIFFERENTIAL DIAGNOSTIC FEATURES OF BOVINE THEILERIAS OF SOUTHERN AFRICA

Species	Schizonts	Piroplasms	Disease+	Serotype
Theileria parva parva	++++	++++	East Coast fever	*T. parva*
Theileria parva bovis	++	+	Rhodesian theileriosis (seasonal)	*T. parva*
Theileria parva lawrencei	+	+/–	Corridor disease (buffalo contact)	*T. parva*
so-called *Theileria mutans* (transmitted by *R. appendiculatus*)	+/–	+	benign	*T. taurotragi*
Theileria mutans (transmitted by *Amblyomma* spp)	+/–*	+	benign	*T. mutans*
Theileria velifera	?	+*	benign	*T. velifera*

+ some or all of these parasites may be responsible for "turning sickness" (cerebral theileriosis)
* morphology distinctive
++++ parasites numerous; + parasites commonly seem in small numbers; +/– parasites sometimes seen.

into *T. parva.* Serological studies by Schindler et al[12] and Lohr & Ross[10] revealed a very close relationship between *T. parva, T. lawrencei* and *T. bovis* and this has been confirmed by other workers. It is currently thought that all three parasites belong to the species *T. parva* but they are distinguishable in the field. Uilenberg has suggested that they should be regarded as subspecies and has introduced the names *T. p. parva* and *T. parva lawrencei*[18]. To these may be added *T. parva bovis.*

The most recent development has been the demonstration that the benign parasite previously known as *T. mutans* consists of at least two species, one (*T. mutans* proper), transmitted by *Amblyomma* species[15] and the other (also called *T. mutans* in southern Africa) transmitted by *R. appendiculatus*[16]. They are clearly distinguishable serologically from each other and from *T. parva.* The *R. appendiculatus*-transmitted parasite may be identical to *Theileria taurotragi*[5]. A third benign species, *Theileria velifera,* has recently been recorded in South Africa[1]. It can be distinguished from the others serologically and morphologically. The distinguishing features of the bovine theilerias are summarised in Table 1.

DIFFERENTIAL DIAGNOSIS

There are three possible approaches to the differential diagnosis of the bovine theilerias.

Serology

The one absolute difference between the various species, on which their modern classification is based, is their serological characteristics. It is not possible to separate the members of the *T. parva* group serologically but one can differentiate this group from *T. mutans,* so-called *T. mutans* and *T. velifera.*
This can be done in two ways:

(a) detection of antibodies in the host. An animal recently recovered from infection will show antibodies detectable by the indirect immunofluorescence (IFA) test, or other serological tests, using an appropriate antigen. This technique can be used for retrospective diagnosis on a recovered case or it may be applied on a herd basis to determine which theileria is present. The only drawback is the relatively short persistence of antibodies detectable by tests currently in use. Burridge and Kimber[3] found that, after experimental *T. p. parva* infection, IFA antibodies to schizont antigen persisted for a mean of seven months, but some animals became negative after three months. The persistence of antibodies to *T. parva bovis* appears to be similar, with an estimated 30 per cent of naturally infected animals becoming negative after six months (Lawrence, unpublished). *T. mutans* antibodies persist longer, for at least one year[7], but as *T. parva* group and *T. mutans* frequently occur together the presence of antibodies to one does not preclude simultaneous infection with the other. Serum antibodies may provide positive evidence of infection but their absence does not exclude the possibility of a parasite being present.

(b) identification of piroplasms. The piroplasms of most species are morphologically indistinguishable but they do react specifically with homologous antibodies and this can be demonstrated using the IFA test. The method has been used to distinguish between *T. p. parva* and *T. mutans* in field infections[13]. To be successful, the parasitaemia in the animal to be examined must exceed 1 per cent[17] and such levels are very uncommon with the members of the *T. parva* group found in southern Africa today, or indeed with the other species of theileria found there. The method is attractive but impractical.

Morphology of the Parasite

Differences in size, appearance and numbers of schizonts and piroplasms have been described between the species and sub-species of theileria. The work of Jarrett et al[6] with the Muguga strain of *T. p. parva,* however, reveals the tremendous variation that can result within one strain when animals are infected at different levels and examined at different intervals after infection. While one may accept that a characteristic morphological appearance can be expected in a typical case of infection with one or other theileria, the morphology of the parasite will do little to clarify a diagnosis in the atypical case. The only theilerias which can be clearly recognised morphologically are *T. mutans,* in which the schizonts are unmistakeably different from those of the other species, and *T. velifera,* in which the piroplasms have a distinctive veil.

Epidemiology

In the final analysis, the differential diagnosis of the bovine theilerias in the field usually depends on various aspects of the epidemiology; the geographical situation, the presence or absence of vectors, the presence or

absence of buffalo, morbidity, mortality, seasonal incidence and the ability to spread. The differences in epidemiology which were the original basis for the classification of the theilerias still remain as the most useful distinguishing features.

CONCLUSION

In the first part of this paper it was demonstrated how the differences between the species and sub-species of *Theileria* in southern Africa came to be recognised. Some of these differences have since been confirmed as being specific, by studies of serology, morphology and life cycle. The differences between the members of the *T. parva* group are not specific, but they are nevertheless recognisable. They appear to be conditioned by environment. Thus, *T. p. parva* is adapted to continuous passage through cattle, the tick vector having a non-seasonal distribution in most enzootic areas. *T. parva bovis* is adapted to seasonal passage through cattle, the tick instars having a strictly seasonal distribution. *T. parva lawrencei* is adapted to buffalo and infects cattle accidentally. In stable conditions the different variants are mutually exclusive, they do not coexist and their differential diagnosis is not a problem. However, they can survive outside their natural location, at least for a period. *T. p. parva* persisted for 50 years in southern Africa after it was introduced. It is in such circumstances that differentiation between the variants becomes important and it is then that it is most difficult, because the variants are not always stable and the difference between them are not absolute. From the practical aspect of the veterinarian working in southern Africa, the important thing is to recognise East Coast fever if it is reintroduced or appears spontaneously, for East Coast fever is more pathogenic and spreads more readily than any of the indigenous theilerias of southern Africa. The main distinguishing features appear to be high morbidity and mortality and the presence of large numbers of schizonts and piroplasms. The other theilerias should not present a serious problem, the pathogenic variants are not difficult to control and misdiagnosis of the occasional atypical case would not be a serious error.

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CURRENT ANAPLASMOSIS CONTROL TECHNIQUES IN THE UNITED STATES

K.L. KUTTLER

ABSTRACT: Kuttler K.L.; **Current anaplasmosis control techniques in the United States.** *Journal of the South African Veterinary Association* 1979 **50** No. 4 314-320 (En) U.S. Department of Agriculture, Science and Education Administration, Animal Parasitology Institute, Beltsville, Maryland 20705.

A card test for detecting anaplasmosis, along with the complement-fixation test, has proved useful in identifying carrier animals. This identification, associated with treatment with tetracyclines, has been a basis for control in the southeast where anaplasmosis is endemic. The tetracyclines are used parenterally (terramycin 11 mg/kg, 10–14 days) or orally (2,2 to 11 mg/kg, 45–60 days).

Notwithstanding these methods, anaplasmosis remains a problem and many animals require treatment to moderate the course of acute infection. A new drug, T-200 (a long-lasting terramycin), has been tested and found effective in treating acute infections (20 mg/kg, 1 time) and in clearing carrier infections (20 mg/kg, 2 times at a 7-day interval).

For several years, a killed adjuvant vaccine was extensively used to control anaplasmosis. The occurrence of neonatal isohemolytic anaemia in association with this vaccine has discouraged its use. This vaccine is still being marketed, but is usually used on selected animals. An attenuated *Anaplasma* vaccine of ovine origin has been developed, but is not licensed for use in the United States although it has been successfully used elsewhere.

INTRODUCTION

Anaplasmosis can be described as an acute or subacute, infectious non-contagious disease of cattle characterized by anaemia, high fever, and icterus caused by an arthropod-transmitted rickettsia, *Anaplasma marginale*.

Since 1910 when Theiler[69 70] first associated the erythrocytic marginal points with a specific and distinct disease, the interest and research in this very widespread and costly cattle disease has been great. Theiler[69 70] considered *A. marginale* to be a protozoan. This theory was generally accepted until recently, when studies presented evidence that suggested a closer relationship to the rickettsia[2 29 30]. At one time, the theory was prevalent that *A. marginale* was a virus[19]. During the last 10–15 years, however, a consensus has been growing in the U.S. that it is probably a rickettsia.

Notwithstanding the hundreds of research papers on anaplasmosis dealing with such subjects as serologic diagnosis, cell-mediated immune response, antibodies, chemotherapy, and vaccines, an absolute control program has yet to be developed. Animals still contract the disease, sicken, and die of the infection.

Anaplasmosis is a severe problem in the U.S. where it is an endemic disease in 3 distinct geographical areas[65]:

1. The southeastern U.S. has a warm, humid, often subtropical climate where investigators believe flying insects, more particularly biting flies, of the family *Tabanidae* are the mechanical vectors.
2. The Intermountain West has a temperate climate with cold, often wet winters with significant snow accumulations and hot dry summers. In these zones, the tick *Dermacentor andersoni* is thought to be the principal biological vector. The anaplasms are transmitted seasonally, usually spring and early summer.
3. The West Coast has a Mediterranean climate, characterized by cool, wet winters and hot dry summers. In this area, the tick *Dermacentor occidentalis* is thought to be the principal biological vector. Unique to this area is the presence of an important wildlife reservoir (black-tailed deer, *Odocoileus hemionus columbianus*) that greatly complicates control efforts[8 52].

Anaplasmosis in the U.S. is not confined to these areas, but it is usually sporadic or of low incidence elsewhere, and can often be traced to imported cattle. Mule deer *(Odocoileus hemionus hemionus)* in the Intermountain area harbor the infection in nature and may act as a reservoir host[57]. White-tailed deer (WTD) *(Odocoileus virginianus)* in the eastern half of the U.S. are susceptible to *A. marginale* experimentally, but have not been found naturally infected[42 43]. They are not thought to be a major reservoir of infection. This apparent paradox of a susceptible host (WTD) that is not infected in a disease endemic zone is not clearly understood and requires greater study.

DIAGNOSIS

One of the most significant contributions to the control of anaplasmosis was the development of the Complement-Fixation (CF) test to detect carrier or chronically infected reservoirs of infection[5 6 23 25 50 51 53 55]. This test was developed in rudimentary form as early as 1934 with antigens derived from infected ticks[56]. Not until later, however, did the test become standardized and accepted as official[5 6]. The CF test was followed by a Capillary Tube-Agglutination (CA)[a] test, and more recently a Rapid Card Agglutination (CT)[b] test[3 4]. The CA antigen is no longer produced commercially, but this technique for detecting either the acute or chronic phase of *Anaplasma* infection is accurate and simple. Antigen preparation has been described[58 74] and the antigen can easily be prepared by research laboratories. The test involves filling capillary tubes with antigen and serum and allowing the tubes to stand on end overnight; during that time, a clear agglutination reaction occurs with serums from *Anaplasma*-positive cattle. A comparison of the CF and CA tests on 501 serums from 4 herds (Table 1) showed an overall agreement of 88,2 %[31]. Comparisons of serum titers from known in-

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a Diamond Laboratories, Des Moines, Iowa.
b Hynson, Westcott, and Dunning, Baltimore, Maryland.

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Table 1: SEROLOGIC RESULTS ON 501 CATTLE FROM 4 HERDS WITH THE COMPLEMENT-FIXATION (CF) AND THE CAPILLARY TUBE-AGGLUTINATION (CA) TESTS[31]

	CF Test			CA Test		%
	Negative	Suspicious	Positive	Negative	Positive	Agreement
	258 *			241	17	93,4
		33		11	22*	66,6
			210	31	179	85,2
Totals	258 (51 %)	33 (7 %)	210 (42 %)	283 (56 %)	218 (44 %)	
Overall agreement						88,2 %

* CF suspicious reactions that were CA positive are considered in agreement.

Table 2: SEROLOGIC RESULTS ON 361 ANIMALS FROM A SINGLE HERD WITH THE COMPLEMENT-FIXATION (CF) AND THE RAPID (PLASMA) CARD AGGLUTINATION (CT) TESTS[40]

	CF Test			CT Test		%
	Negative	Suspicious	Positive	Negative	Positive	Agreement
	46			42	4	91,3 %
		41		8	33*	80,5 %
			274	16	258	94,2 %
Totals	46 (13 %)	41 (11 %)	274 (76 %)	66 (18 %)	295 (82 %)	
Overall agreement						92,2 %

* CF suspicious reactions that were CT positive are considered in agreement

fected cattle tested with both the CF and CA showed significant correlation, with a probability of error of < 0,01 %. A comparison of the CT and CF test was conducted on 361 plasma and serum samples. The CT was run on 361 heparinized plasma samples at the ranch the day of bleeding and the CF test was later conducted in the laboratory on serum samples from the same animals. These tests showed a 92,2 % agreement. The results are summarized in Table 2.

The CT is available for official use in the U.S., but is not distributed commercially. The CT is easily performed in the laboratory on serum or as a field test on heparinized plasma with results available within minutes after blood collection. In performing the test, a drop (0,03 mℓ) of serum or plasma is placed on a card to which is added a drop (0,03 mℓ) of normal bovine serum factor and a drop (0,015 mℓ) of antigen[3]. The serum, normal bovine serum factor and antigen are stirred with a toothpick or similar object. The card is then placed on a rotator for 4 minutes. Agglutination results are read immediately. Ambient temperature is important. Temperatures should range from 21°C to 27°C. Plasma can be tested immediately, but serum should be allowed to stand 48 hours at approximately 24°C for greater accuracy[3 4].

With the development of these diagnostic tests,[41] a system of control evolved which consists of testing a herd, identifying the reacting or carrier stock, and removing the source of infection by: (1) slaughter; (2) segregation and isolation of infected cattle; or (3) treatment of infected animals to eliminate the carriers[61]. Several monthly re-tests are generally required, depending on vector activity and the rate of transmission. Although this approach has been successfully used in the U.S., it is most practical in areas of low incidence or marginal transmission and is most often attempted on an individual farm basis at owner expense. Because slaughter is seldom elected and segregation is often impractical, the method of choice has more frequently been treatment to eliminate the herd reservoir of infection[11].

TREATMENT

Until the last few years, the only specific chemotherapeutic compounds have been the tetracyclines, principally oxytetracycline,[c] chlortetracycline,[d] and tetracycline hydrochloride[e] [39]. These tetracyclines all appear similar in suppressing the reproduction of *Anaplasma* organisms. Foote and co-workers[18] described this action in 1951, but noted that chlortetracycline was not effective when given late in the course of infection. The desirability of using the tetracyclines early in the course of infection was noted by others[15 48 49].

Soon after the tetracyclines were observed to inhibit growth of *Anaplasma,* experiments were conducted to evaluate tetracyclines against carrier infections[10 11 20 22 47 54 61 68]. The experiments indicated that carrier infections could be eliminated but only after the prolonged administration of fairly large doses. Nevertheless, this significant breakthrough stimulated a great deal of research with the ultimate development of numerous successful treatment regimes (Tables 3 and 4) that effectively eliminate carrier infections[39]. At the present time, 1 of 2 approaches is generally recommended: administer either chlortetracycline orally at the rate of 11 mg/kg daily for 45–60 days or oxytetracycline intravenously or intramuscularly at the rate of 11 mg/kg daily for 10–12 days.

A relatively new experimental formulation of oxytetracycline (T–200)[f] has advantages over previous oxyte-

c Oxytetracycline: Liquamycin, Terramycin (50 mg/mℓ), Charles Pfizer and Co., Inc.
d Chlortetracycline: Aureomycin, Lederle Laboratories, American Cyanamide Co.
e Tetracycline hydrochloride: Polyotic, Lederle Laboratories, American Cyanamide Co.
f T–200: Terra/long-acting 200 mg oxytetracycline/mℓ, Charles Pfizer and Co., Inc.

Table 3: SUCCESSFUL TREATMENT REGIMES FOR THE ELIMINATION OF *ANAPLASMA* INFECTION WITH THE TETRACYCLINE DRUGS[39]

Drug	Rate of Administration	Route	No. of Treatments	Interval	Reference
Tetracycline	11 mg/kg	IV or IM	10	Daily	Pearson et al[54]
Oxytetracycline	11 mg/kg	IV or IM	12–14	Daily	Splitter and Miller[68]
Chlortetracycline	33 mg/kg	IV	16	Daily	Splitter and Miller[68]
Chlortetracycline	2,2 mg/kg	Orally	41	Daily	Franklin et al[22]
Chlortetracycline	5,5 mg/kg	Orally	45	Daily	Franklin et al[22]
Chlortetracycline	1,1 mg/kg	Orally	120	Daily	Franklin et al[21]*
Chlortetracycline	11 mg/kg	Orally	30–60	Daily	Franklin et al[24]
Chlortetracycline	11 mg/kg	Orally	60	Daily	Brock et al[11]
Chlortetracycline	5,5 mg/kg	Orally	60	Daily	Brock et al[11]
Chlortetracycline	3,3 mg/kg	Orally	60	Daily	Brock et al[11]
Chlortetracycline	11 mg/kg	Orally	45–60	Daily	Roby et al[61]
Chlortetracycline	11 mg/kg	Orally	30	Daily	Twiehaus[72]
Oxytetracycline	22 mg/kg	IV	5	Daily	Magonigle et al[47]
Oxytetracycline (T-200)	20 mg/kg	IM	2	7 day	Roby et al[64]

IV = Intravenous; IM = Intramuscular.
* Negative Status Determined by Serologic Procedures

Table 4: TREATMENT REGIMENS USING OXYTETRACYCLINE PLUS GLOXAZONE, IMIDOCARB AND IMIDOCARB PLUS GLOXAZONE FOR THE ELIMINATION OF *ANAPLASMA* INFECTION[39]

Drug	Rate of Administration	Route	No. of Treatments	Interval	Reference
Oxytetracycline*	11 mg/kg	IV	3	1 or 2 days	Kuttler[35 36 37]
+					
Gloxazone	5 mg/kg				
Imidocarb*	5 mg/kg	IM or SC	3	Daily	Kuttler[36]
Imidocarb*	2 mg/kg	IM or SC	3	Daily	Kuttler[36]
+					
Gloxazone	5 mg/kg				
Imidocarb**	5 mg/kg	IM or SC	2	14 days	Roby et al[63]

IV = Intravenous; IM = Intramuscular; SC = subcutaneous
* Splenectomized calves tested. ** Adult cattle tested

tracycline[c] formulations[44]. It contains 200 mg/mℓ of oxytetracycline whereas the older formulation contains 50 mg/mℓ. The new product also establishes more sustained blood levels and has been successfully used to clear infection from carrier animals with as few as 2 injections at a 1-week interval (Table 3)[64].

Compound T–200 is not yet cleared for use in the U.S.; but if and when it is, control procedures using T–200 will undoubtedly become an attractive alternative because of the simpler regimen and increased efficacy[44 64].

Two new compounds, Gloxazone[g] and imidocarb[h], have recently been described as having a specific chemotherapeutic effect on *Anaplasma*[7 26 28 35 36 39 40 60 62 63]. In premunization experiments[45], Gloxazone (5 mg/kg) and imidocarb (4 mg/kg) were superior to oxytetracycline (11 mg/kg) in moderating the course of infection in adult cattle intentionally exposed to virulent *A. marginale.*

Neither Gloxazone nor imidocarb has been approved for use in the U.S. Both Gloxazone and imidocarb are occasionally toxic[1 36] The LD_{50} for imidocarb in cattle was determined to be 15 mg/kg given intramuscularly 2 times at a 2-week interval, for a total dose of 30 mg/kg (Personal communication Drs L.G. Adams and D.E. Corrier, Dept of Veterinary Pathology, Texas A & M University, College Station, Texas). In normal usage (2–5 mg/kg), the compound is rarely toxic; but when doses are increased to clear infection, the toxic range is approached. The usual route of administration for imidocarb is either subcutaneously or intramuscularly. Intravenous inoculations can be hazardous[36]. The route for Gloxazone is usually intravenous.

g Gloxazone: (356C61). Alpha-Ethoxytethylglyoxal Dithiosemicarbazone, Burroughs Wellcome Co.
h Imidocarb: (4A65). Imizol, 3,3'bis-(2-imidazolin-2-yl)-carbanilide dihydrochloride (or dipropionate), Burroughs Wellcome Co.

IMMUNIZATION

In highly endemic areas, treatment to eliminate infection has been considered by some to be contra-indicated because of the high risk of reinfection and the relative vulnerability of the herd with no substantial carrier immunity. For this reason, methods of immunization have been developed to protect susceptible animals from acute infections.

Within a year of his description of *A. marginale,* Theiler described a second organism, *Anaplasma centrale*[71], which produced mild infections that were immunologically related to the more virulent *A. marginale*[32 33]. *A. centrale* was subsequently used as a vaccine and is still in use[14]. The persisting infections produced by *A. centrale* or *A. marginale* referred to as premunition have been the basis for anaplasmosis immunization for years[67].

Table 5: MEAN RESPONSE OF ADULT CATTLE TO PREMUNITION WITH ATTENUATED *ANAPLASMA MARGINALE, ANAPLASMA CENTRALE,* AND VIRULENT *ANAPLASMA MARGINALE*[38]

Premunizing organism	No. of animals	Average Pre-Infection PCV (%)	Average Incubation time (days)	Average low PCV (%)	Average high parasitemia (%)	Average duration of anemia (days)	Deaths
Attenuated *A. marginale*	14	34,4	25,1	24,9	3,40	8,6	0
A. centrale	14	36,5	17,1	26,5	4,04	8,3	0
Virulent *A. marginale*	18	32,9	22,6	14,5	12,10	26,6	2
Significance*		NS	NS	P<0,01	P<0,01	P<0,01	
DRS				5,4	5,0	11,6	

PCV = packed cell volume; NS = not significant; DRS = difference required for significance.
* An analysis of variance was conducted on values obtained for the 3 *Anaplasma* groups

Table 6: MEAN RESPONSE OF INTACT CALVES TO PREMUNITION WITH ATTENUATED *ANAPLASMA MARGINALE, ANAPLASMA CENTRALE,* AND VIRULENT *ANAPLASMA MARGINALE*[38]

Premunizing organism	No. of animals	Average age (months)	Average Pre-Infection PCV (%)	Average Incubation time (days)	Average low PCV (%)	Average high parasitemia (%)	Average Duration of anaemia (days)
Attenuated *A. marginale*	10	5,1	31,0	29,3	22,0	1,56	6,4
A. centrale	6	2,3	34,8	10,8	23,5	5,60	5,0
Virulent *A. marginale*	17	3,2	33,1	15,4	19,5	7,10	25,5
Significance*		NS	NS	P<0,01	NS	NS	P<0,05
DRS				6,7			19,0

PCV = packed cell volume; NS = not significant; DRS = difference required for significance.
* An analysis of variance was conducted on values obtained for the 3 *Anaplasma* groups.

The relative resistance of young calves to anaplasmosis encouraged the practice of inoculating calves with blood from *Anaplasma*-recovered cows to induce mild infections that are usually followed by recovery and a carrier state with solid immunity[67]. More recently, frozen stabilates of virulent *A. marginale* have been prepared, titrated for infectivity, checked for safety and purity of infection, and used to appropriate dilutions to produce moderate infections that may or may not require suppressive treatment, depending upon animal age and natural resistance[38 45].

In 1968, an attenuated, sheep-adapted, live *A. marginale* vaccine was reported which produced mild, almost non-apparent infection, but induced substantial, long-lasting immunity[59 73]. The relative mean responses in adult cattle and calves to the attenuated *A. marginale, A. centrale,* and virulent (lab-passaged) *A. marginale* are given in Tables 5 and 6[38 45].

The only *Anaplasma* vaccine presently approved for use in the U.S. is prepared from a blood-based antigen combined with adjuvant*[9]. This vaccine is not infective and produces a distinct but low level of resistance to anaplasmosis. It was and is still being successfully used in some areas. A few years after wide-scale use of this vaccine, neonatal isohemolytic anaemia (NIA) was associated with the use of this vaccine in some calves[17]. The erythrocyte stromal antigens in the vaccine apparently elicited antibodies in some cows. Depending upon calf blood type, colostral antibodies appeared responsible for inducing severe anaemia in calves. Many of these calves died. Those that survived and apparently recovered were more susceptible to *Anaplasma* infection[75]. Experimentally induced infections in such calves showed that they were as susceptible as splenectomized calves. Surgery on NIA-recovered calves showed severe splenic atrophy. Spleen weights from these calves recovered from NIA averaged only 0,053 kg; those from normal calves of comparable size averaged 0,6 kg.

Table 7 presents a summary of various immunizing systems, with a subjective evaluation of use and efficacy based on our experience under field and laboratory conditions.

The sheep-adapted attenuated vaccine has been safe and highly effective in our hands[45]. There are, however, reports of reversion to virulence[34]. Cattle vaccinated with this agent become carriers of infection so cannot be certified as disease-free on animal health certificates, and accordingly cannot be shipped interstate. The vaccine has been tested extensively in the U.S., but it has not been licensed for marketing.

VECTOR CONTROL

Vector control as a means of controlling or eradicating arthropod-borne hemoparasites such as *Babesia, Theileria,* and *Trypanosoma* has proved unusually successful. Although work along these lines has been very limited for *Anaplasma* control, the possibility exists and obviously should receive greater attention. Hoffman and coworkers reported that the dissemination of anaplasmosis within a susceptible herd can be reduced through intensive insect control[27].

The fact that *Anaplasma* may be transmitted both biologically and mechanically by a large number of arthropod vectors has led most workers to assume that approaches other than vector control are more desirable. However, the need for vector control is well illus-

* Anaplaz: Ft. Dodge Laboratories, Ft. Dodge, Iowa.

Table 7: SYSTEMS OF ANAPLASMOSIS IMMUNIZATION

	Calves < 8 months old	Cattle 8 months to 2 years old	Cattle > 2 years old	Relative efficacy
Virulent field *Anaplasma marginale*	Safe NR	Not safe NR	Not safe NR	++++
Virulent field *Anaplasma marginale* with therapy	Safe NR	Safe NR	Not safe NR	++++
Dilute stabilate	Safe R	Safe NR	Not safe NR	++++
Dilute stabilate with therapy	Safe R	Safe R	Safe R	++++
Attenuated *Anaplasma marginale*	Safe NR*	Safe R	Safe R**	+++
Anaplasma centrale	Safe R	Safe R	Safe R**	++
Killed vaccine	Safe NR	Safe NR	Safe R	+

R = recommended; NR = not recommended
* The mildness of this organism is such that a satisfactory replicating infection is not always produced in calves of this age.
** Not recommended for lactating dairy cattle.
++++ Maximum protection against needle and field challenge.
+++ Solid protection against needle challenge. Variable response against some field challenges.
++ Partial protection against both needle and field challenge. prevents death losses by either challenge.
+ Partial protection against needle challenge. Will usually prevent death losses against a moderate challenge. Imposes NIA hazards in producing cows.

trated by an experiment in which a herd of 469 cattle with over 80 % anaplasmosis incidence was treated with imidocarb at levels that should have eliminated infection[40]. All cattle were treated, but no vector control was instituted. The initial response to treatment was favorable, and the incidence of serologic reaction decreased to less than 40 % within 40 days, with no evidence of clinical infection. However, within 1 year, the herd incidence returned to the 80 % level. This gradual increase in rate of infection among treated animals paralleled the increase seen in *Anaplasma*-negative controls in the herd.

Our studies of natural transmission have been badly neglected over the years, and for this reason, we do not always recognize which vectors are primarily responsible. Furthermore, the epizootiology of anaplasmosis is complicated by unsanitary management practices such as dehorning, castration, or multiple use of hypodermic syringes and needles.

DISCUSSION

The time and expense of treatment with presently available drugs has discouraged the use of test-and-treatment techniques in large-scale efforts to eradicate anaplasmosis. The killed vaccine, once widely recommended, is now administered more cautiously to only bulls and selected animals because of NIA problems. *Anaplasma centrale* has never been allowed in the U.S., and the failure to license the sheep-adapted attenuated *A. marginale* vaccine has for the present eliminated this product as an effective tool in the control of anaplasmosis.

Low-level feeding of tetracyclines to susceptible cattle during the vector season is a common practice. The chemoprophylactic effect of feeding low levels of the tetracycline drugs has been described[12 16 21 22 66 72]. Investigators found that as little as 1,1 mg/kg fed daily would prevent acute anaplasmosis. Possibly even lower levels may be effective.

A common practice followed by many veterinary practitioners in the Southeast is to wait until the first case of anaplasmosis occurs or until the first animal dies, then treat the entire herd with a parenteral tetracycline, and repeat this treatment in 4–6 weeks. In following this practice, it is believed that cases in the incubative stages will be aborted, that transmission will be retarded, and that the second treatment will protect the herd until the vector season draws to a close. This emperical approach to a serious problem leaves much to be desired, but it is an available tool for the practicing veterinarian and livestock producer.

Prospects for an improved, scientifically sound control program would be greatly increased when and if the new oxytetracycline formulation (T-200) becomes available. We are now testing other synthetic tetracyclines that show promise and some other slow-release compounds that might enable us to clear infections with a single injection. Work is also underway to reduce humoral antibody response but to enhance cell-mediated response with our present killed vaccine, and thus eliminate or reduce the NIA problem[13].

In conclusion, we can safely say that our knowledge of the problem is extensive, and the tools to combat infection are formidable; nevertheless, the disease persists and losses continue. Even though we can control and eradicate infection on a local or small-scale basis, conditions are not yet suitable for starting wide-scale or area eradication programs for anaplasmosis.

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EXPERIMENTAL THERAPY OF THEILERIOSIS

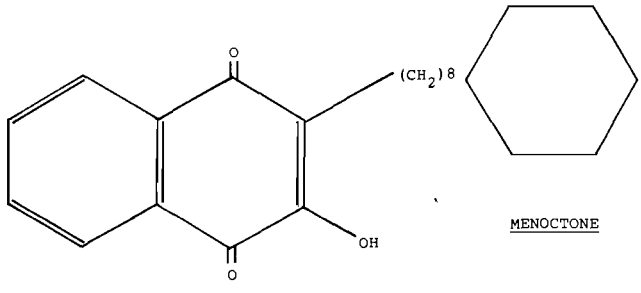
N. McHARDY

ABSTRACT: McHardy N.; **Experimental therapy of theileriosis.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 321-322 (En). Department of Parasitology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

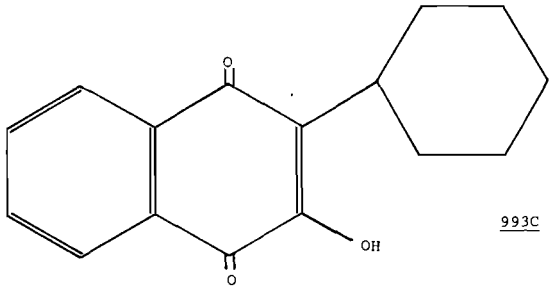
A tissue culture method was used to screen compounds for activity against *Theileria parva,* and demonstrated that the hydroxy-alkylated naphthoquinones, 'menoctone' and 993C were highly active, with ED_{50} values around 0,005 mg/ℓ. When injected into cattle artificially infected with *T. parva,* menoctone cured all of 7 cattle at a total dosage of 10 mg/kg injected intravenously (i.v.). A further trial showed that injection of menoctone, 10 mg/kg, as a single dose by the intramuscular (i.m.) route was more effective than when a similar dose was given by the i.v. route. Titration of serum from these cattle in the *in vitro* system showed that inhibitory levels of drug persisted for three days after i.v. injection and six days when menoctone was injected i.m. The minimum effective dose level of 993C was 20 mg/kg i.m. either as a single dose, or as two doses of 10 mg/kg with an interval of 48 hours.

INTRODUCTION

Attempts at discovering a drug with which to treat theileriosis began about 70 years ago, in South Africa, and a major effort was made from 1950–1965, both in South Africa[4] and East Africa[6]. These studies demonstrated that the tetracycline antibiotics can prevent the establishment of theileriosis and may exert some therapeutic effect too. The other major finding was that none of a very large number of candidate compounds exerted any useful effect in the therapy of theileriosis. In 1975 we discovered that the antimalarial compound 'menoctone' had a very marked effect on *Theileria parva* and *Theileria annulata in vitro,* and later showed that this compound was very effective in the therapy of experimental East Coast fever – *T. parva* infection[3]. Since then, we have investigated a large number of hydroxy-alkylated 1:4-naphthoquinones related to menoctone, and have selected one, compound '993C', for further development. In this paper I will describe some of our results obtained with menoctone and 993C, whose availability has enabled us to investigate some of the basic problems in the treatment of theileriosis.



MENOCTONE



993C

EXPERIMENTAL RESULTS

Our work on the therapy of theileriosis is based on the culture system for *T. parva* developed by Malmquist, Nyindo and Brown (1970)[2]. Experimental compounds are incubated for 48 hours in the presence of bovine lymphoblastoid cells infected with *T. parva.* Smears are then made and the stained cells are examined to observe the effect of the compound.

In control cultures, almost 100 % of the cells contain schizonts. When menoctone and 993C were tested at 0,1 mg/ℓ, practically all the schizonts were destroyed, and the lymphoblastoid cells appeared to be reverting to a small lymphocytic form.

Menoctone is difficult and very expensive to produce, largely because of the difficulty of attaching the 8-carbon-atom side-chain directly to the naphthoquinone moiety. In compound 993C the 8-carbon-atom side-chain is omitted and the cyclohexyl ring is attached (Fig.). We hope to be able to produce this compound at an economical price.

Compound 993C is very highly active *in vitro,* with an ED_{50} value of about 0,005 mg/ℓ. Prolonged exposure of cultures to the compound at low concentrations does not appear to induce resistance to the effect of the compound, but an anti-parasitic effect can be seen at concentrations of 993C as low as 0,00001 mg/ℓ. At 0,1 mg/ℓ 993C has a very rapid effect on both the percentage of cells which contain a recognisable schizont (after 48 hours, only about 15 % of cells contain a schizont), and also on the number of chromatin particles in each schizont, which falls from around 10 to less than two particles.

The mode of action of these compounds is not certain, but may be related to an effect on electron transport, via the synthesis or functioning of co-enzyme Q, as suggested for malaria parasites by Peters (1974)[5]. The effect seen *in vitro* is that the schizonts become clearly disorganised and usually less strongly stained, before total disappearance, sometimes leaving a 'scar' in the host cell.

In our early studies in cattle artificially infected with *T. parva,* we injected menoctone by the intravenous route. The work was carried out at Muguga, Kenya, in association with the FAO Project on Tick-borne diseases, under the leadership of Dr M.P. Cunningham. Dr T.T. Dolan was most closely associated with this work. The cattle in all the trials were infected by the injection of a stabilate of ground up infected tick suspension (GUTS) as described by Cunningham *et al* (1973)[1].

In the first experiment, fourteen calves were infected and observed for the development of symptoms of theileriosis. On the first day on which schizonts could be observed in lymph-node smears and rectal temperature was 39,5°C or more (day TS), seven received menoc-

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tone, 5 mg/kg i.v. then 1 mg/kg i.v. for each of the next five days – a total dose of 10 mg/kg. Seven were left untreated. Six of these controls died while the disease was rapidly cured in all seven treated cattle, which were subsequently shown to be resistant to homologous challenge.

In a second experiment carried out at the Wellcome Kenya Laboratories, Kabete, with Dr D.G. Rae, five cattle treated with exactly half this dose regimen of menoctone – 2,5 mg/kg + 5 × 0,5 mg/kg i.v. – total 5 mg/kg, also recovered rapidly. Five cattle which received a single dose of 5 mg/kg i.v. on day TS only, responded less well, and although they all recovered, the treatment was clearly less effective than the multiple-dose regimen. Only two of five controls died in this experiment.

It seems, therefore, that for effective therapy of ECF, effective concentrations of menoctone need to be present for a sustained period of time. Although a single large dose of drug had a more rapid and dramatic immediate effect, it often permitted a recrudescence of the infection, sometimes severe, about a week after treatment,

The effect of the drug on the parasites seen in lymph node biopsy material was similar to that seen in cultured cells. The normal morphology of both macroschizonts and microschizonts was severely disrupted within 24 hours of treatment. In animals with piroplasms in the erythrocytes, menoctone had an equally rapid and pronounced effect on this stage of the parasite.

Results so far, therefore, indicated that menoctone injected i.v. is effective in the therapy of ECF but that it must be given daily for several days for the effect to be greatest. We next tried to prolong the effect of menoctone by administering it by routes other than i.v. Groups of 5 steers were given menoctone, either by mouth or by intramuscular injection for comparison with the effect obtained by the i.v. route. The cattle were treated with a single dose of 10 mg/kg menoctone on day TS3, the third day of temperature and schizonts, when the animals were clearly becoming sick.

Four untreated controls died, while one became severely sick and recovered. Treatment by mouth had practically no effect on the infection apart from a transient effect on temperature. The cattle treated by the i.v. and i.m. routes all recovered but, while those treated by the i.v. route showed a recrudescence about 5 days after treatment, the infection was rapidly controlled in the cattle injected by the i.m. route. A similar clear effect was seen in the weight-changes in these animals.

By titrating serum from treated cattle in the *in vitro* system, we showed that the compound had persisted at inhibitory concentrations for six days following i.m. injection but only three days after i.v. injection. Only a trace of activity was found 24 hours after administration in the cattle dosed by mouth. These findings correlate well with our clinical observations.

At this time, compound 993C was shown to be as active as menoctone in the *in vitro* system. Our preliminary trial in cattle showed that it, too, was most effective when injected i.m.

In a further experiment to find the minimum effective dose of 993C, groups of 5 cattle received 20, 10 or 5 mg/kg as a single dose injected i.m. on day TS5, when the cattle were clearly sick with ECF. The 20 mg/kg dose was the most effective treatment, saving all five cattle, while three of five untreated controls died, one became severely ill but recovered, and the fifth developed only mild ECF. Following treatment with 993C at 10 mg/kg i.m. one animal died, while two died at 5 mg/kg. The course of the infection was very similar to that seen following treatment with menoctone.

In a further experiment with 993C, we gave cattle two doses of 10 mg/kg i.m. on days TS4 and TS6. While all 5 untreated controls died the five which received 993C all made rapid recoveries, and had regained their initial weight by three weeks after treatment.

Titration of serum from individual cattle in this experiment showed a close correlation between effect of treatment and concentration and persistence of 993C in the serum.

CONCLUSION

We therefore have, in 993C, a compound which we hope to develop for the treatment of ECF and, hopefully, also other *Theileria* infections. Perhaps equally important, we now have a tool which enables us to study the effects of therapy on ECF, and which also makes possible a wide range of experiments on the immunology and biochemistry of theileriosis. These investigations are beginning now in several research establishments, and we hope that they will lead to a better understanding of, and therefore better methods of controlling, theileriosis.

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FIELD EXPERIENCE WITH HEARTWATER *(COWDRIA RUMINANTIUM)* IN CATTLE

LENTE VAN DER MERWE

ABSTRACT: Van der Merwe, Lente; **Field experience with heartwater *(Cowdria ruminantium)* in cattle.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 323-325 (En) P.O. Box 10, Settlers 0430, Republic of South Africa.

Observations are reported on 2743 animals immunised against heartwater since 1958. The methods of immunisation and control of reactions are discussed. This procedure is not without risk and 0,83 % of the animals died in spite of treatment.

Bos indicus breeds appear to have a greater resistance to heartwater and are relatively easy to immunise. Of the *Box taurus* breeds the South Devon, the Jersey and the Charolais seem to be particularly susceptible.

Older animals and pregnant cows are considered to be in the high risk category.

INTRODUCTION

Heartwater is highly prevalent in the Bushveld areas of the Northern Transvaal and bovines introduced to the area from heartwater free areas are at grave risk.

Neitz & Alexander[3] showed that a sulphonamide, Uleron was of chemotherapeutic value against heartwater when given during the febrile reaction period of this disease. Later, Weiss et al[6] and Haig et al[2] showed that the antibiotics chlortetracycline and oxytetracycline were more effective drugs, and recommended that both antibiotics be given early in the course of the febrile reaction before other clinical symptoms appeared. Recovered animals were found to have a solid immunity against heartwater.

The need to immunise valuable stock being introduced into the heartwater infected areas of the Northern Transvaal encouraged the author to develop facilities to do this immunisation.

The work was commenced in 1958 and continued to date.

METHOD

The method of immunisation was essentially that developed by Neitz & Alexander[3]. The *Cowdria ruminantium* infected blood used in all animals was the Ball 3 strain issued by the Veterinary Research Institute – Onderstepoort. From 1958 to 1968 a single dose of 5–10 ml blood was administered intravenously. Since 1969 two doses of 5–10 ml were given 5–7 days apart.

Rectal temperatures of all animals were taken morning and evening from the day of inoculation until animals were discharged. When morning temperatures above 39,5°C or evening temperatures above 40,0°C were recorded, the animals were treated (ambient temperatures were taken into consideration before treatment).

The treatment given was oxytetracycline by the intramuscular route. Initially the dose was 2 mg/kg. Over the years this was increased gradually. Today the dose is 10 mg/kg using a 100 mg/mℓ concentrate. Treatment was repeated after 24 hours, irrespective of the temperature response of the animal. If the temperature persisted for longer than 48 hours or other clinical symptoms appeared a further dose of tetracycline was given intravenously. A 33,3 % sulphadimidine sodium solution was given intravenously with the oxytetracycline when severe symptoms persisted.

A total of 2743 animals were immunised.

RESULTS

The procedure is not without risk and out of 2743 animals presented for immunisation 24 or 0,83 % died in spite of treatment (Table 1).

An analysis of the results (Tables 2, 3, 4 and 5) shows that breed, sex, age and group size are important considerations.

Study of the temperature charts, maintained for each animal, shows that they can be divided into three distinct categories, a typical reaction in two thirds of cases, (Figure 1), a persistent temperature reaction (Figure 2) and a twin peak reaction (Figure 3).

Table 1: REACTION TO HEARTWATER IMMUNISATION

Number of cattle	Non-reactors	Died	Died after leaving the centre
2743	7	24 (0,83 %)	5*

*Diagnosis of heartwater not confirmed by a veterinarian in 3 cases.

Table 2: RISK ACCORDING TO SEX

	Numbers	Died	% Mortality
Bulls	1050	6	0,57 %
Pregnant Cows	822	11	1,34 %
Cows and Heifers	871	7	0,8 %
	2743	24	

Table 3: RISK ACCORDING TO AGE

	Numbers	Died	% Mortality
Younger than 8 years	2709	20	0,74%
Older than 8 years	34	4*	11,7%
	2743	24	

*One bull developed permanent blindness and had to be destroyed.

Table 4: INFLUENCE OF GROUP SIZE

	Numbers	Died	% Mortality
Groups greater than 50	526	12	2,2 %
Lesser groups or individuals	2181	12	0,5 %
	2743	24	

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Table 5: RISK ACCORDING TO BREED

Breed	Numbers	Died	% Mortality
Afrikander	579	0	–
Bonsmara	183	0	–
Brahman	648	8	1,2
Santa Gertrudis	327	2	1,0
Simmentaler	303	3	1,0
South Devon	118	4	3,3
Charolais	42	1	2,5
Hereford	51	1	2,0
Friesland	394	3	0,75
Jersey	72	2	3,0
Other	26	0	–
Total	2743	24	

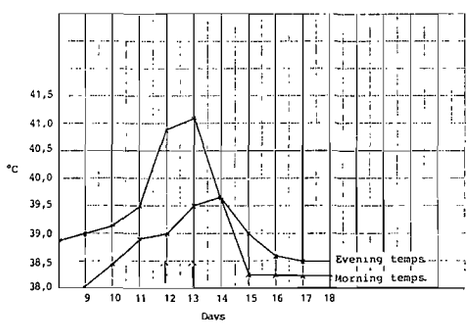

Fig. 1. Typical heartwater reaction as seen in two thirds of cases. Treated on Days 12 and 13 evenings (arrows).

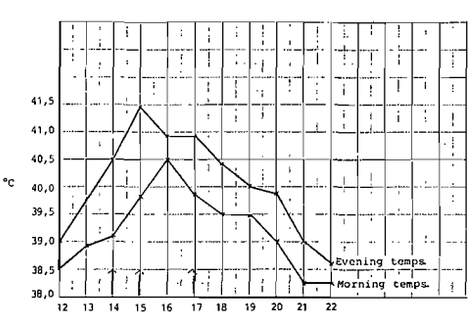

Fig. 2. Persistent temperature reaction. Treated on Days 14, 15 and 17 (arrows).

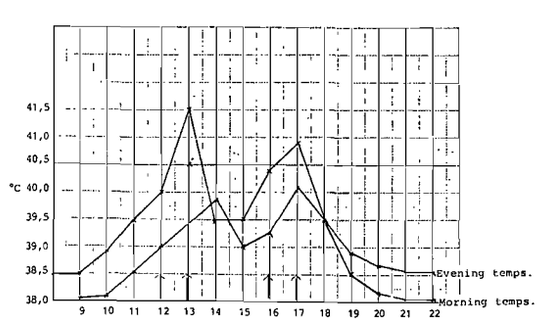

Fig. 3. Twin Peak Reaction. Treated on Days 12, 13 16 and 17 (arrows).

DISCUSSION

The practice of giving two inoculations of infected blood 5–7 days apart was introduced because animals who failed to react to the initial dose and who were given a second infecting dose 30 days later, frequently developed anaphylactic shock.

The severity of the reactions in the cattle varied significantly, possibly because the virulence of the *Cowdria* organisms may vary from passage to passage in sheep.

Reactions to infection with sheep passaged *Cowdria* infected blood usually start about the twelfth day, the range lying between the 9th and 30th day. The majority of reactions fall between the 12th and the 19th day.

Care must be taken in evaluating the temperature reactions as ambient weather conditions can play an important role. On winter mornings animals may not show a rise in temperature, but develop other clinical signs shortly after the temperature was read. Equally in summer, evening body temperatures can be misleadingly high especially if cattle have been upset or driven.

Regular and thorough clinical examinations are essential especially over the period when the temperature reaction is expected i.e. from the 9th day after infection. About 3 % of animals develop visible clinical signs before showing a temperature reaction. The commonest of these signs are petechiae in the mucous membranes of the eye and vagina, hypersensitivity of the eye reflex and oedema of the lungs. Such animals should be treated immediately and kept in a cool dark room under intensive care. The mortality rate amongst these animals was higher than amongst those showing normal temperature reactions.

Treatment was administered routinely by the intramuscular route. When a high temperature persisted for longer than 48 hours or when other clinical symptoms appeared, more oxytetracycline was given intravenously. Shock may follow this administration and the animals should be closely watched. A 33,3 % sulphadimidine sodium solution administered intravenously with the oxytetracycline dose gives good results when severe symptoms persist.

Oxytetracycline is highly irritant when given intramuscularly and this irritation can cause an already hypersensitive animal to exhibit severe and sudden nervous symptoms. Furthermore, it can cause swellings and such swellings may persist and become a blemish in a show animal.

The risk of immunising pregnant cows is high (Table 2) and is directly proportional to the stage of pregnancy. Heavily pregnant animals are prone to show severe clinical symptoms. Oedema of the lungs often develops in such animals and suggests a poor prognosis.

Older animals are also in a higher risk category (Table 3). Animals over 8 years of age are at a very high risk and immunisation of such aged animals should be approached with caution.

A very important influence on the success or otherwise of immunisation is the level of the management available. Large groups are very difficult to manage successfully and from bitter experience a maximum group size of 50 animals has been established.

The *Bos indicus* breeds appear to have a greater resistance to heartwater and are relatively easier to immunise than the *Bos taurus* breeds (Table 5). Having

said that, the eight deaths in the Brahman group need explanation. Of these, seven were from a group of one hundred and seven animals which were immunised at the same time. Four were heavily pregnant and developed lung oedema. The whole group was exceptionally wild and difficult to manage and heavy and persistent rain further complicated the situation. Effective management is very difficult under such conditions and the exercise is dangerous.

Of the *Bos taurus* breeds the Simmentaler and the Friesland are relatively easy to immunise because they are easy to handle, show typical symptoms and respond well to treatment.

The South Devon, the Jersey and Charolais seem to be particularly susceptible and losses were not exceptional. These breeds react severely to the infection and require careful nursing.

With a typical reaction, that is, when the animal presents a typical temperature chart (Figure 1), only two treatments 24 hours apart are given.

Indeed, one treatment would suffice in a majority of cases and the second dose is given as a safety precaution.

When a subject exhibits a persistent temperature reaction (Figure 2) a third treatment is invariably given, but by the intravenous route. The necessity for this third dose is debatable, but because of the value of the animals under treatment no unnecessary chances can be taken.

The twin peak reaction has been described by other workers[2,4,5] and here four treatments are given (Figure 3). Again, the need for repeated treatments has not been proved. However, the regime works as is shown by the low losses incurred over the years.

Under field conditions it is very difficult to assess the efficacy and persistence of the immunity obtained. To date only five instances of a breakdown in immunity have been reported to the author of which three were confirmed by positive brain smears.

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PRELIMINARY OBSERVATIONS ON THE COMBINED USE OF IMIDOCARB AND *BABESIA* BLOOD VACCINE IN CATTLE

R.J. TAYLOR and N. McHARDY

ABSTRACT: Taylor R.J.; McHardy N. **Preliminary observations on the combined use of imidocarb and *Babesia* blood vaccine in cattle.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 326-329 (En). Kwanyanga Res. Stn, Coopers (SA) (Pty) Ltd, P.O. Box 5034, 5208 Greenfields, Cape Province, Rep. of South Africa.

Imidocarb was used by three different methods to control reactions in cattle induced by a *Babesia* blood vaccine produced in South Africa.

Simultaneous administration of 0,15 mg/kg imidocarb and *Babesia bovis* vaccine gave satisfactory control. When the vaccine was given seven days prior to the imidocarb treatment a dose between 0,15 mg/kg and 0,6 mg/kg imidocarb was required for effective control.

A combined *B. bovis* and *Babesia bigemina* vaccine given at 21 and again 61 days after a 3 mg/kg imidocarb treatment allowed the development of an adequate premunity to both these parasites.

INTRODUCTION

The Redwater blood vaccine currently produced in South Africa contains live forms of both *Babesia bovis* and *Babesia bigemina*. This vaccine is capable of producing severe clinical reactions in some animals and users are advised to take the temperature of vaccinated animals daily and to treat those which show clinical symptoms with specific drugs.

A method of controlling the clinical reaction and eliminating the need to temperature animals daily while at the same time allowing the development of immunity would greatly increase the acceptability of the vaccine. To this end, de Vos[4] advocated the use of diminazene 7 days after inoculation of *Babesia* blood vaccine to control the reaction. Callow and McGregor[2] described the vaccination of cattle with *Babesia argentina* during chemoprophylaxis with a quinuronium compound which reduced the risks of vaccination and did not interfere with the development of acquired immunity. Callow and McGregor[3] and Brown and Berger[1] demonstrated the activity of imidocarb in cattle against various *Babesia* species including *B. argentina* and *B. bigemina*, while the chemoprophylactic effects were described by Roy-Smith[6].

The trials reported here were aimed at investigating the feasibility of using imidocarb to control post-vaccinal reactions without loss of immunity or alternatively to exploit its chemoprophylactic property to attain the same end.

EXPERIMENTAL WORK

Forty-nine 11–18 month old Hereford cross cattle from a redwater free area were used. The redwater vaccine contained the Onderstepoort strain of *B. bovis* and/or *B. bigemina*. *Babesia* strains used for challenge purposes were produced in splenectomised calves and consisted of either the homologous strain or a virulent field strain of either *B. bovis* or *B. bigemina*.

The imidocarb used in this trial was formulated as a 120 mg/mℓ solution of the dipropionate salt.

Clinical response to the vaccine was measured by recording rectal temperatures and PCV and by the examination of Giemsa-stained thick and thin blood smears for *Babesia* organisms. With animals whose peripheral blood smears had remained negative throughout the observation period, brain biopsy was taken[5], stained with Giemsa and examined for *Babesia* organisms.

Experiment 1: Simultaneous vaccination and treatment with imidocarb

Materials and methods

Seventeen cattle which had received 1 mℓ of *B. bovis* vaccine subcutaneously in the neck were divided into three groups of 4 and one group of 5 animals. Three of the groups were injected intramuscularly in the rump with 0,05, 0,15 or 0,3 mg/kg imidocarb. The fourth group was maintained as an untreated control. All the groups were subjected to homologous challenge by intravenous inoculation of 10^8 *B. bovis* infected red cells 45 d after vaccination.

Results and conclusions

The results are summarised in Table 1.

All control cattle and those which received 0,05 or 0,15 mg/kg imidocarb became infected with *B. bovis*. Two of the 4 cattle which received 0,3 mg/kg imidocarb were also infected. This indicated that sterilisation of the infection had occurred in the remaining two animals in this group.

All animals were immune to challenge 45 d after vaccination, except the two animals in which a carrier status could not be demonstrated.

Patency results indicated that there was no difference between the control group and the group which received 0,05 mg/kg imidocarb. This dose rate, therefore, would not be sufficient to control the expected clinical reactions experienced in the field.

A dose rate of 0,15 mg/kg imidocarb given simultaneously with the *Babesia* vaccine gave satisfactory control without sterilising the infection.

Experiment 2: Treatment with imidocarb or diminazene 7 d after vaccination with *B. bovis*

Material and methods

Sixteen cattle were divided into four groups of 4 animals, and inoculated with *B. bovis* vaccine as in Experiment 1. Two groups were injected intramuscularly with either 0,15 or 0,6 mg/kg imidocarb 7 d after vaccination. A third group received 3,5 mg/kg diminazene aceturate* and one group was left untreated as a control. All animals were bled prior to vaccination and 25 d

* Berenil: Hoechst

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Table 1: EFFECT OF SIMULTANEOUS ADMINISTRATION OF IMIDOCARB AND *BABESIA BOVIS* BLOOD VACCINE

Group No.	Dose rate of Imidocarb dipropionate mg/kg	Rectal temperature reaction	Effect on PCV	Treatment of babesia reaction	Animal No.	*B. bovis* Parasitaemia: Blood smear	*B. bovis* Parasitaemia: Brain biopsy	Prepatent period in blood smears (days)	Establishment of carrier status No. of animals	Response to challenge 45 days after vaccination
1	Untreated Control	Nil	Nil	No	1	+	NC	15	4/4	Immune
					8	–	+	ND		Immune
					358	+	NC	15		Immune
					386	+	NC	13		Immune
2	0,05	Nil	Nil	No	2	+	NC	13	5/5	Immune
					5	+	NC	14		Immune
					9	+	NC	15		Immune
					357	+	NC	17		Immune
					475	+	NC	12		Immune
3	0,15	Nil	Nil	No	3	–	+	ND	4/4	Immune
					6	+	NC	20		Immune
					10	–	+	ND		Immune
					385	–	+	ND		Immune
4	0,3	Nil	Nil	No	4	–	–	ND	2/4	Clinical babesiosis
					7	–	–	ND		Clinical babesiosis
					11	–	+	ND		Immune
					470	+	+	15		Immune

ND – Not determined
NC – Not conducted

Table 2: THE EFFECT OF DOSING WITH IMIDOCARB OR DIMINAZENE SEVEN DAYS AFTER INOCULATION WITH *B. BOVIS* BLOOD VACCINE

Group No.	Drug and dose rate	Rectal temperature reaction	Effect on PCV %	Treatment of control animals	Animal No.	*B. bovis* parasitaemia: Blood smear	*B. bovis* parasitaemia: Brain biopsy	Prepatent period in blood smears (days)	Establishment of carrier status No. of animals	Serology T+25
1	Untreated Control	Mild From T+5–T+9 Mean Max 39,7°C	Slight decrease from T+10–T+14	Not necessary	21	+	NC	11	4/4	+ve
					22	+	NC	7		
					23	+	NC	8		
					24	+	NC	7		
2	Diminazene 3,5 mg/kg	Mild From T+6–T+9 Mean Max 39,9°C	No change	N.a.	25	–	+	ND	4/4	+ve
					26	+	NC	24		
					27	–	+	ND		
					28	+	NC	27		
3	Imidocarb 0,15 mg/kg	Mild From T+6–T+10 Mean Max 39,8°C	No change	N.a.	29	+	NC	7	4/4	+ve
					30	+	NC	18		
					31	+	NC	12		
					32	+	NC	14		
4	Imidocarb 0,60 mg/kg	Mild From T+6–T+9 39,8°C	No change	N.a.	33	–	–	ND	3/4	+ve
					34	+	NC	18		
					35	+	NC	17		
					36	+	NC	17		

N.a. – Not applicable
NC – Not conducted
ND – Not determined

after vaccination, and their serum was examined for the presence of antibodies by the IFA test.

Results

The results are summarised in Table 2. All the control animals and those which received diminazene or 0,15 mg/kg imidocarb became infected with *B. bovis*. No parasites could be demonstrated in one of 4 animals which received 0,6 mg/kg imidocarb but the animal was found to be serologically positive. When the vaccine was given seven days prior to the imidocarb or diminazene, a dose rate of 3,5 mg/kg diminazene or 0,15 mg/kg – 0,6 mg/kg imidocarb was required for effective control without sterilisation of the infection.

Experiment 3: Immunity following *Babesia* vaccine administered 21 to 61 d post chemoprophylaxis with imidocarb

Material and methods

Sixteen cattle were divided into four groups of 4 animals. Groups 2, 3 and 4 were injected intramuscularly with 3,0 mg/kg imidocarb on the day of chemoprophylactic treatment (designated T). Group 1 served as the untreated control and was vaccinated on T+21 d with *B. bovis* vaccine and on T+61 d with *B. bigemina* vaccine.

Group 2 received *B. bovis* vaccine only on T+21 d.

Group 3 received *B. bigemina* vaccine only on T+61 d.

Group 4 received *B. bovis* plus *B. bigemina* vaccine on T+21 d and again on T+61 d. Where peripheral blood smears and brain biopsy smears proved negative for *Babesia* parasites, blood from these animals was subinoculated into splenectomised calves in order to determine their carrier status. Animals were bled pre- and post-vaccination for serology and challenged by intravenous inoculation of blood containing 10^8 infected red cells originating from a field strain of *B. bovis* and/or *B. bigemina*.

Results and conclusions

The results are summarised in Table 3. Group1. All animals in the control group became infected with *B. bovis* given on T+21 d. They showed a good (3+) antibody titre to *B. bovis* and were solidly immune to needle challenge. Following the *B. bigemina* vaccine given on T+61 d, all 4 became carriers. Two out of 4 became clinically ill and required therapy with Euflavine**. Good antibody titres were demonstrated and all animals were solidly immune to needle challenge.

Group 2. When *B. bovis* vaccine was given on T+21 d, infection was established in 3 out of 4 animals. Serological results showed that 3 out of 4 had good antibody titres to *B. bovis*, whilst the fourth animal was negative. All remaining animals were solidly immune to needle challenge carried out on T+146 d, one animal died of unrelated causes between the time of sampling for serology and needle challenge.

Group 3. When *B. bigemina* vaccine was given 61 d after treatment with imidocarb, there was no clinical reaction and all animals became carriers of *B. bigemina*. Satisfactory antibody titres were observed in all animals but one and all animals resisted needle challenge with virulent *B. bigemina*.

Group 4. The bivalent vaccine given on T+21 d had been damaged during handling and proved non-viable. When the bivalent vaccine was given again on T+61 d, 2 out of 4 animals showed mild pyrexia (due to *B. bovis*), but did not require therapy.

All the animals were shown to be infected with *B. bovis* while 3 of 4 were also infected with *B. bigemina*.

Table 3: THE EFFECT OF IMMUNITY AND CLINICAL REACTIONS TO BABESIA BLOOD VACCINE ADAMINISTERED 21 AND 61 DAYS POST-CHEMOPROPHYLAXIS WITH IMIDOCARB

Group No.	Imidocarb dose mg/kg Day T	Vaccination	Rectal temperature reaction	Effect on PCV	Treatment of vaccine reaction	Animal No.	*Babesia* spp. parasitaemia: Blood smear	Brain biopsy	Sub-inoculation of blood	Pre-patent period in blood smears (days)	Establishment of carrier status No. of animals	Serology	Response to challenge
							(b)						
			3/4 showed			1	+	NC	NC	12		3+	Immune
1	Undosed	*B. bovis*	mild	Nil	No	2	+	NC	NC	12	4/4	3+	Immune
	control	T+21 d	temperature			3	+	NC	NC	10		3+	Immune
			rises			4	+	NC	NC	7		3+	Immune
							(b)						
						6	–	–	–	ND		±	Immune
2	3,0	*B. bovis*	Nil	Nil	No	8	+	NC	NC	28	3/4	3+	died
		T+21 d				10	+	NC	NC	21		3+	Immune
						16	+	NC	NC	14		3+	Immune
							(B)						
						7	+	NC	NC	12		+	Immune
3	3,0	*B. bigemina*	Nil	Nil	No	9	+	NC	NC	11	4/4	2+	Immune
		T+61d				11	+	NC	NC	25		4+	Immune
						15	+	NC	NC	9		3+	Immune
		B. bovis +				5	–*	NC	NC	ND			
4	3,0	*B. bigemina*	Nil	Nil	No	12	–	NC	NC	ND	0/4	Negative	Na
		T+21 d				13	–	NC	NC	ND			
		(1st vaccination)				14	–	NC	NC	ND			
							(B)						
			2/4 showed	Group		1	+	NC	NC	7		4+	Immune
1	Undosed	*B. bigemina*	temperature	mean	2/4	2	+	NC	NC	7	4/4	3+	Immune
(cont)	control	T+61 d	*rises*	showed *slight*	treated with	3	+	NC	NC	7		4+	Immune
				depression	Euflavine	4	+	NC	NC	7		4+	Immune
							(b) (B)	(b)	(b) (B)	(b) (B)	(b) (B)	(b) (B)	(b) (B)
		B. bovis +				5	– +	..	+ +	ND 10		2+ 2+	Immune Immune
4	3,0	*B. bigemina*	2/4 showed	Nil	No	12	+ –	NC	+ –	17 ND	4/4 3/4	3+ +	Immune Immune
(cont)		T+61 d	mild			13	– +	+	NC	ND 12		3+ 2+	Immune Immune
		(2nd vaccination)	temperature rises			14	+ +	NC	NC	11 9		3+ 2+	Immune Immune

Na: Not applicable
ND: Not determined
NC: Not conducted
–: negative
+: positive

(B): *Babesia bigemina*
(b): *Babesia bovis*
* Infectivity of vaccine destroyed in transit

** Centaur Labs

Serology showed good antibody titres to *B. bovis* but the response to *B. bigemina* was minimal.

All animals were solidly immune to needle challenge with *B. bovis* and *B. bigemina* field strains.

Results in Group 2, 3 and 4 indicate that the combined *B. bovis* and *B. bigemina* vaccine, given 21 d and again 61 d after injection of imidocarb allowed the development of the carrier state and consequently immunity to both *B. bovis* and *B. bigemina*, and also reduce the risk of severe clinical reactions to the vaccine.

DISCUSSION

In the three experiments, the *B. bovis* vaccine proved to be an ideal blood vaccine, as it produced only mild clinical reaction and good immunity.

Experiment 1 showed that when animals that had not developed the infection following vaccination were needle challenged with the homologous strain, they became clinically ill. This indicated that the animals were fully susceptible to a challenge dose of *B. bovis*. In the absence of clinical symptoms in both the controls and imidocarb treated animals, assessment of the control of clinical reactions by the drug were restricted to the consideration of the length of pre-patency and the degree of difficulty with which the parasitaemia could be demonstrated. Parasites were usually demonstrated in peripheral blood smears of control animals but with imidocarb treated cattle, brain biopsies and subinoculation of blood was needed on occasion.

The *B. bigemina* vaccine proved to be a more virulent vaccine and produced clinical symptoms requiring therapy in 2 out of 4 control animals. In experiment 3 the serological results obtained for *B. bigemina* following inoculation with the heterologous vaccine appeared to be slight, especially as false positive reactions to *B. bigemina* occur as a result of immunity to *B. bovis*. However, these animals proved to be solidly immune to subsequent needle challenge.

Simultaneous vaccination and treatment (experiment 1) offers the advantage of the single handling of cattle, reduces drug level variables and gives rise to less concern about the stage of development of the infection. In contrast the regimen adopted in Experiment 2 necessitates handling the cattle twice and there is less control over the stage of development of the infection at the time of treatment.

In experiments 1 and 2 the dose of imidocarb was directed only at *B. bovis*. If it is accepted that had the vaccine also contained *B. bigemina*, this fraction would have been eliminated, and immunity only to *B. bovis* would have resulted.

In experiment 3 the chemoprophylactic properties of imidocarb were combined with the use of the bivalent *Babesia* vaccine. This system has the advantage that, immediately following the injection of imidocarb, the animals would be protected from babesiosis from 4 weeks and after the two doses of bivalent vaccine, would be immunized against both parasites. The first dose of bivalent vaccine given on T+21 d would confer premunity to *B. bovis*. Imidocarb prophylaxis against *B. bigemina* is generally longer than that afforded against *B. bovis*. In fact, it is expected that in most animals the *B. bigemina* fraction of the vaccine would be eliminated after the first vaccination. However, there would be sufficient chemoprophylactic activity remaining against *B. bigemina* to prevent clinical infection from natural field challenge until the premunity had been developed to *B. bigemina* following the vaccination on T+61 d. The *B. bovis* fraction would at that time act as a booster to the *B. bovis* premunity.

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THE IMMUNE RESPONSE OF CATTLE TO LIVE AND INACTIVATED ANAPLASMA VACCINES AND RESPONSE TO CHALLENGE

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INTRODUCTION

The nature of various aspects of the immune response to anaplasma infection has been described by numerous investigators. Globulin levels are elevated[1] and several serologic tests[2 3] have been used to detect the presence of specific antibodies. There is evidence of an autoimmune response during acute anaplasmosis which is at least partly responsible for the characteristic anemia[4]. Cell mediated immunity (CMI) has been described as an important element of the protective immune response[5] and is therefore an essential component to be elicited by an immunogen.

It has long been recognized that protection against virulent *A. marginale* can be afforded by premunization using homologous *A. marginale*[6] or induction of heterologous immunity by establishment of *A. centrale* infection[7]. Use of an anaplasma stabilate of measured minimal dosage more safely offers effective protection compared to traditional methods of premunization[8 9]. An inactivated commercial anaplasma vaccine induces a certain degree of protection[10] but has been related to neonatal isoerythrolysis (NI) in newborn calves from vaccinated dams[11]. The attenuated anaplasma vaccine of ovine origin[12], commercially available in Latin America, has been proven to be reasonably effective and safe[13]. The mechanism of action of each of these types of immunogens has been the subject of research on measurement of the cell mediated response to infection and vaccination.

IMMUNE RESPONSE TO LIVE AND KILLED IMMUNOGENS

The leukocyte migration inhibition test (LMIT) and lymphocyte transformation (LT) test[13] were used as *in vitro* correlates of cellular immunity and compared to *in vivo* functions measured by delayed cutaneous hypersensitivity[15] and challenge[5]. The capillary agglutination (CA) and complement fixation (CF) tests have been routinely used to determine the humoral response.

Virulent *A. marginale*

The LMIT response increased above background levels about 4 weeks after infection, remained elevated for 1–4 months and then gradually declined over the remainder of the 9 month observation period[14]. The CA test first became positive 3 to 4 weeks after infection. Acute clinical signs of anaplasmosis were concurrent with evidence of parasitemia and decreased hematocrit. These cattle were strongly immune to challenge with virulent anaplasma[5].

Cattle which responded to virulent anaplasma with a detectable increase in the LMIT level prior to the onset of hematologic and clinical signs usually showed relatively mild acute disease and readily recovered from initial exposure. Conversely, cattle which did not show this early evidence of cellular response often became severely ill and died[5].

Attenuated *A. marginale*

The LMIT became positive 2.5 to 3 weeks after inoculation and followed a pattern similar to that described for cattle which received virulent anaplasma. The CA test became positive after 3 to 4 weeks. There were no clinical signs of illness, relatively low levels of parasitemia and only a mild hematocrit reduction below normal[14]. Animals in this group were immune to challenge but some evidence of parasitemia developed after challenge[5].

Inactivated *A. marginale*

Only a transient and low level LMIT response was detected after cattle received 2 inoculations of commercially available killed vaccine[a] in adjuvant. The CA test became positive 2–4 weeks after the first injection. No parasitemia, hematocrit change or clinical signs occurred[14]. These cattle were protected against development of a high post challenge parasitemia but anemia and concomitant signs of clinical illness followed inoculation with virulent anaplasma[5].

A mild LT response was detected using leukocytes from cattle which received either virulent or attenuated anaplasma[14]. When cattle were inoculated with 2 injections of the killed preparation in adjuvant this response was more pronounced.

ISOANTIBODY RESPONSE

Intradermal skin tests using antigen prepared from normal and anaplasma infected bovine and ovine erythrocytes were applied in cattle which had received the various anaplasma immunogens[15]. Animals which had received killed anaplasma in bovine erythrocytes were sensitive to bovine and ovine erythrocyte antigen but animals which were inoculated with killed anaplasma in ovine erythrocytes were sensitive only to ovine erythrocyte antigen.

Hemagglutination and hemagglutination lysis tests using normal ovine and bovine erythrocytes indicated that there was a strong antibody response to bovine erythrocytes which resulted from the use of killed anaplasma vaccine of bovine origin. Little or no antibody against bovine erythrocytes was detected in cattle which received the ovine origin immunogen.

CHEMICALLY MODIFIED ANAPLASMA ANTIGEN

One method of avoiding development of anti-erythrocyte antibody may be by chemical modification of the

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[a] Anaplaz. Fort Dodge Co., Fort Dodge, Iowa

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antigens to preclude induction of humoral antibody while permitting normal or enhanced development of CMI[16]. This approach presupposes the greater importance of the cellular response in protection. Two methods of chemical treatment were chosen for use. The first, lipid conjugation, employs dodecanoic acid (DA)[17]. The other involves an electrostatic binding of dimethyl dioctadecyl ammonium bromide (DDA) to antigen[18].

Antigenic modification of the killed vaccine using DA greatly reduced the isoantibody titers which developed to selected bovine blood group substances subsequent to vaccination[17]. However, chemical treatment did not drastically reduce the cell mediated response as detected by the LT. Anti-anaplasma antibody detected by the CF test was induced by the unaltered antigen but not by the modified antigen. Both groups of cattle responded similarly to challenge but since it has been postulated that protection is related to humoral and cellular immunity absence of CF antibody in cattle inoculated with modified vaccine was considered due to an undesirable effect of chemical treatment.

Dimethyl dioctadecyl ammonium bromide allowed stimulation of anti-anaplasma CF antibodies while largely reducing development of anti-bovine erythrocyte isoantibody[18]. Both the unaltered and modified antigens induced similar levels of cellular immunity, indicated by the LT test, and offered similar protection against challenge. Therefore, the DDA method of chemical modification was noted to retain the essential effect of the killed vaccine while reducing the potential for NI.

DISCUSSION

The ovine origin attenuated anaplasma vaccine offers protection which approaches the somewhat more absolute immunity afforded by premunization with virulent anaplasma. Both procedures stimulate a protective response probably involving antibody, lymphocytes and macrophages. Inactivated vaccine produces a lesser degree of protection while producing undesirable isoantibody. Use of a procedure for chemically modifying the anaplasma antigen may increase its value in situations where immunization is indicated but use of a live agent is prohibited.

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VIRULENCE AND IMMUNOGENICITY OF CULTURED *THEILERIA ANNULATA* SCHIZONTS

E. PIPANO*

Schizonts of *Theileria annulata* were cultured *in vitro* for the first time about 35 years ago. However, it was only after growing this parasite in monolayer cell cultures, around the 1960's that the use of cultured schizonts for immunization of cattle was considered.

Internal organs, like liver, spleen, or lymph nodes from *T. annulata* infected animals, have been used to initiate cultures. However, lymphocytes in the peripheral blood of cattle in the acute stages of the infection are in practice the most reliable source for material for primary cultivation of this parasite.

Under optimal conditions about 50 % of primary cultures may transform to *Theileria* infected cell lines. Feeder cells are not necessary for the establishment of cultures but if such cells are present a higher rate of transformation is obtained.

The infected lymphoid cells grow either in suspension or as a monolayer depending upon how the initial passages are carried out. It appears that all cells in the cultures contain schizonts as demonstrated by fluorescent antibody and other staining techniques.

Two types of dividing cells can be observed in established cultures. Those undergoing mitotic division and others showing binary fission of the nucleus followed by the division of the cytoplasm. In both types of division the schizont is shared by the two daughter cells.

Partial or complete attenuation of the virulence of *T. annulata* schizonts can be achieved by cultivation *in vitro*.

Parasites from low cell culture passages behave like schizonts from blood passages when they are inoculated into susceptible cattle. They produce clinical theileriosis during which schizonts are easily detected in lymph-node and liver biopsy material. Later erythrocytic stages appear in peripheral blood.

Further cultivation diminishes the virulence of the parasites. Inoculated cattle show milder clinical signs, but both schizonts and erythrocytic parasites can still be found.

When complete attenuation is reached no clinical signs follow inoculation of culture schizonts and no parasites can be detected in lymph nodes, liver smears or blood films.

Some strains of *T. annulata* may continue to produce schizonts when inoculated into cattle even after 300 or more passages in culture, but the erythrocytic stages never appear. However, if heavy stress occurs simultaneously, clinical theileriosis may result in cattle infected with such schizonts.

There is no indication that the time needed for attenuation is proportional to the initial virulence of the schizonts. No satisfactory explanation can as yet be provided for the phenomenon of attenuation. It seems that schizonts grown for relatively long periods *in vitro* lose the capacity to complete their normal life cycle in cattle. Such schizonts undergo a limited multiplication in cattle without attaining the number needed to provoke clinical disease or reaching the stage of microschizonts and producing erythrocytic parasites.

It appears that attenuated schizonts have the same immunological features as virulent schizonts since immunization with living attenuated schizonts confers total protection against virulent homologous schizonts. Antibody response of cattle to attenuated schizonts is similar to that induced by virulent schizonts. However, it should be stressed that resistance to reinfection in theileriosis is not dependent on the level of circulating antibody.

A question that is very relevant to immunization is the immunogenic relationship between the developmental stages of *T. annulata*. At least three stages in the life cycle of *T. annulata* can produce infection in cattle: mature parasites from tick salivary glands, macroschizonts and erythrocytic stages.

Cross protection between schizonts and erythrocytic stages seems to be non-existent even when living parasites are used for immunization. On the other hand the immunological relationship between schizonts and infective stages derived from ticks has a considerably greater practical importance. The stage specific immune response concerning these two developmental forms is well demonstrated in immunization with killed schizonts. When such schizonts are inoculated together with adjuvant they may engender total protection against virulent schizonts, but not the slightest protection against tick induced infection.

Immunization with living schizonts gives protection against infection by ticks since the parasites inoculated by ticks transform to schizonts in cattle. However, this resistance is almost never complete and immunized animals show some parasites or even fever after inoculation of theilerial stages derived from ticks. One possible explanation for this might be that immunity against *Theileria* acts mainly before the lymphocytic stage of the parasite. The few tick-parasites that survive the initial immune response and succeed in penetrating lymphocytes may then be inaccessible to the host's protective response.

The immunity induced by living schizonts compared with infective *Theileria* stages from ticks was assessed in cattle immunized with: (a) various numbers of attenuated schizonts; (b) virulent schizonts, and (c) attenuated schizonts, followed by virulent schizonts.

Upon challenge with infected ticks most of the above cattle showed a mild rise in body temperature accompanied in some cases by a few parasites in the liver. All animals survived. Cattle recovered from this tick transmitted infection were totally protected against further homologous challenge. On the other hand, 9 susceptible control calves showed heavy clinical theileriosis and 4 of them died.

An important feature of *T. annulata* schizonts with regard to immunization is their high infectivity for cattle.

Blood – transmitted *T. annulata* invariably infects

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cattle even when relatively low doses are used. Four out of four calves became infected following inoculation of 1 mℓ of blood drawn during the acute stage of theileriosis from a donor in which no schizonts could be seen on examination of blood smears.

In a recent experiment cattle were inoculated with virulent schizonts from low cell culture passages. Doses of 10^3 and 10^4 schizonts caused infection in 3 out of 4 calves, and 10^5 or more schizonts infected all inoculated animals.

In a similar experiment infectivity of attenuated cultured schizonts for cattle was evaluated. As such schizonts do not provoke clinical symptoms or parasitaemia in cattle the response to inoculation was assessed by measuring serum antibody levels and also by challenge with homologous virulent schizonts. Animals that received 5×10^3 schizonts did not develop antitheilerial antibodies. One of the two calves that received $2{,}5 \times 10^4$ schizonts and all animals that received 10^5 schizonts showed antibody titres. On challenge animals that were serologically negative were also susceptible, while the remaining were totally protected.

Vaccine prepared from attenuated schizonts for immunization in the field is used in two forms: as a suspension of freshly grown schizonts in Eagle's MEM, which is stored and transported at 4°C and has a shelf life of 3–4 days; or as concentrated suspension of schizonts frozen in pellets or vials. The frozen vaccine is stored and transported in liquid nitrogen and has a shelflife of more than one year. The material is thawed and diluted in the field and inoculated within 30 minutes of thawing.

These vaccines are completely safe for all breeds and types of cattle, including pregnant dairy cows. They give sufficient protection to enable local and exotic breeds to survive in *T. annulata* enzootic areas.

The degree of protection varies with the age and breed of animal. According to present experience young calves (up to 6–7 months) of all breeds withstand tick infection if they have been immunized previously with the cell culture vaccine. The immunity produced by the vaccine will actually be reinforced by exposure to infected ticks in the field.

Adult cattle of most breeds are protected by the vaccine to about the same degree as the young animals – with one exception – the adult Friesian dairy cow, which even when vaccinated may suffer clinical symptoms, reduced milk production, and, if pregnant, abortion following infection by ticks in the field.

Reinoculation of such animals with virulent schizonts considerably improves their resistance to tick transmitted infection. Even stronger immunity can be achieved by reinoculation with frozen stabilate of ground *Theileria* – infected ticks.

Although many phenomena associated with growth *in vitro,* virulence and immunity in *T. annulata* still await elucidation, the cultivation of this parasite has been a great stride forward in control of theileriosis. Among the various possible systems for immunization, the cell culture vaccine appears at present to be the most reliable method for application in the field.

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INVESTIGATIONS ON THE NATURAL AND ACQUIRED RESISTANCE OF CATTLE TO ARTIFICIAL INFECTION WITH *COWDRIA RUMINANTIUM*

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ABSTRACT: Du Plessis, J.L.; Bezuidenhout, J.D. **Investigations on the natural and acquired resistance of cattle to artificial infection with *Cowdria ruminantium*.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 334-338 (En) Veterinary Research Institute, Onderstepoort, Rep. of South Africa.

Sixty cattle, artificially immunized against *C. ruminantium*, were challenged 3, 6, 12 and 24 months later. Levels of conglutinin were determined in pre-immunization serum samples. There appeared to be a relationship between the pre-infection levels of conglutinin and the susceptibility of the animals to heartwater: At the time of immunization 31 out of 60 cattle (51,6 %) with levels in the low-titre range (1:10–1:160) showed a typical febrile response, whereas 29 (48,4 %) with conglutinin levels in the high-titre range (1:320 and higher) were either fully or partially resistant.

Except for 2 animals that developed mild febrile reactions when they were challenged 2 years after artificial immunization, all the cattle were fully resistant to challenge with *C. ruminantium* infected sheep blood. It can be concluded that in this experiment cattle retained their immunity to heartwater for at least 2 years in the absence of reinfection.

INTRODUCTION

Variation in the susceptibility to *Cowdria ruminantium*, not only of different breeds of cattle[16] and sheep[14], but also in individual animals of the same breed of cattle[13], has been recognized for a long time. In a preliminary report, Neitz and Alexander[12] stated that some evidence has been obtained that pure-bred individuals of the exotic breeds (Aberdeen Angus and Hereford) are more susceptible than grades. Following the immunization of a group of pure-bred Afrikaner cattle, these workers subsequently concluded that the pure-bred Afrikaner is no more resistant to heartwater than the exotic breeds mentioned above[13].

Whereas practically 100 % sheep from heartwater free areas react to artificial infection with the Ball 3 strain of *C. ruminantium*[18], it is common experience that only 60–70 % of cattle raised in heartwater-free areas show a reaction when submitted to artificial immunization[16,17]. As a result of this, a second injection of infective blood is recommended for all animals which do not react to the first[3].

Conglutinin, a serum protein found only in ruminants[10], disappears from and subsequently reappears in the serum of cattle infected both with *Anaplasma marginale*[15] and *Ehrlichia phagocytophila*[11], both of which are classified as rickettsial agents in the order *Rickettsiales*[2]. This observation prompted the authors to investigate the levels of conglutinin in the serum of cattle infected with *C. ruminantium*.

Long-term experiments on heartwater immunity in significant numbers of cattle under laboratory conditions can be expensive. The use of cattle on an experimental farm therefore has advantages, but also limitations if the farm is situated in a heartwater enzootic area. An experiment originally designed to study the duration of immunity to *C. ruminantium* in cattle, therefore not only afforded an opportunity to assay conglutinin levels but also to determine the feasibility of carrying out heartwater research on cattle maintained under extensive management on a farm practically exempt from the arthropod vector, in a heartwater enzootic area.

MATERIALS AND METHODS

Animals

Afrikaner-Simmenthaler-cross heifers 1–2 years old were kept at pasture on a Government experimental farm situated in a heartwater enzootic area. Regular weekly dipping had been practised for several years before the experiment and was maintained during it. Tick infestation was minimal.

Monitoring of natural infection by ticks

At monthly intervals during the course of the experiment the adult *Amblyomma hebraeum* ticks on 6 animals chosen at random were collected. From 6 months after the commencement of the experiment the number of animals examined was increased to 16. The ticks were either allowed to feed on susceptible sheep or ground in buffered lactose peptone* and inoculated intravenously into sheep. Sheep that survived were challenged with infected sheep blood (Ball 3 strain) one month later.

Experimental procedure

A single batch of frozen sheep blood, infected with the Ball 3 strain of *C. ruminantium* and issued by the Onderstepoort Veterinary Research Institute for the immunization of domestic ruminants was used and administered intravenously to the cattle both during the immunization and challenge procedures. The dose was 10 mℓ in each case. Rectal temperatures were recorded early each morning and animals which showed a temperature of 40,5°C or higher on 2 consecutive days were treated with oxytetracycline at a dosage of 10 mg/kg bodyweight. Animals that failed to react or that showed mild febrile reactions only were given a second inoculation of 10 mℓ heartwater infected blood 8 weeks later.

Three, 6, 12 and 24 months after immunization, groups of 15 animals and 5 uninoculated controls were challenged as described above. Fifteen days after the challenge inoculation blood was collected in heparin from one animal that had shown a pronounced reaction, one whose reaction was mild, and one that had shown no reaction at all when they were initially immunized. Each sample of blood was inoculated intravenously into a susceptible sheep in order to detect a possible subclinical infection. This procedure was repeated 18 days after challenge using the same sheep.

*Buffered lactose peptone: 181 g Na_2HPO_4; 26,4 g KH_2PO_4; 30 ℓ distilled water, 2 % Difco peptone, 10 % lactose.

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The temperatures of the sheep were recorded daily and they were challenged with heartwater infected blood (Ball 3 strain) one month later.

Blood smears, prepared at the height of the febrile reaction from those animals showing a rise in temperature, were stained with Giemsa and examined for the presence of intercurrent infections with *Babesia* and *Anaplasma*.

Serum samples were collected from all animals prior to the immunizing inoculations and stored at –20°C, until they were submitted to the conglutinin test. To assay conglutinin levels in sheep, serum samples were also collected from 10 Merino sheep prior to being inoculated with heartwater infected blood.

Assay of conglutinin

Conglutinin levels were measured by a modification of the method described by Lachmann[10].

The test was carried out in 6 steps:

1. Rabbit haemolytic serum*, a rabbit anti-sheep red blood cell (RBC) serum, was diluted to 1:500 by the addition of 0,02 mℓ of antiserum to 10 mℓ of complement-fixation test (CFT) diluent**. The solution was then inactivated at 56°C for 30 minutes.
2. Ten mℓ of the diluted, inactivated haemolytic serum was mixed with 1 mℓ of 10 % washed sheep RBC. The mixture was left to stand for 1 hour at room temperature.
3. Subsequently 1,75 mℓ of the sensitized RBC suspension was mixed with 0,025 mℓ horse serum and 3 mℓ CFT diluent. The mixture was then incubated at 37°C for 30 minutes.
4. Meanwhile, 0,5 mℓ of the serum to be tested for conglutinin was inactivated at 56°C for 30 minutes and subsequently adsorbed by the addition of 0,025 mℓ of 10 % washed sheep RBC. The suspension was incubated at 37°C for 30 minutes and the cells sedimented by centrifugation at 1500 r.p.m.
5. Twofold serum dilutions in CFT diluent were prepared from the adsorbed serum and 0,2 mℓ of the serum dilutions added to 0,2 mℓ sensitized sheep RBC bearing fixed complement and the mixtures incubated at 37°C for 30 minutes.
6. The sheep RBC were centifuged to the bottom of the tubes, resuspended by gentle shaking and the degree of agglutination estimated. The titre was expressed as the highest dilution of serum that agglutinated the 3-part complex.

The χ^2 method[8] was used in the statistical evaluation of the relationship between the conglutinin titres and the severity of the reactions shown by the cattle to inoculation with the heartwater agent.

RESULTS

Reactions following immunization

The reactions of the cattle to *C. ruminantium*-infected sheep's blood were arbitrarily divided into 4 categories according to the severity of the clinical manifestations.

The results are summarized in Table 1. Category 1 represents those animals which exhibited a severe reaction manifested by a rise in body temperature to at least 40,5°C for 3 consecutive days or longer and clinical signs of anorexia and depression. The clinical response of 9 of the 60 cattle (15 %) fell into this category. The average incubation period, duration of the febrile reaction and the height of the fever reaction in this group were 14 and 6 days and 41°C respectively.

Animals in category 2 showed a marked febrile response which was unaccompanied by other clinical signs. Twenty-two (36,7 %) of the animals showed febrile reactions which could be classified in this category. Average incubation periods, duration of the febrile reaction and the height of the temperature reaction in this group were 14,7 and 5,1 days and 41°C respectively.

Category 3 involved 18 animals (30 %) which only exhibited mild transient febrile reactions of less than 40,5°C in single or multiple peaks. The average incubation period in this group was 15,4 days, the average duration of the febrile reaction 2,4 days and the average maximum temperature 40,3°C.

Eleven animals (18,3 %) in category 4 failed to react. Three out of the 29 animals in categories 3 and 4 which were given a second inoculation, responded with pronounced febrile reactions and 2 of them had to be treated. The incubation period in case of all 3 was 14 days, the duration of the febrile response varied between 4 and 8 days and the height of the fever reaction between 40,8 and 41,5°C.

Ten sheep inoculated with the same pool of infected blood all reacted severely with an average incubation period and duration of febrile reaction of 9,4 and 5,2 days respectively and an average maximum temperature of 41,9°C

Assay of conglutinin

In the statistical evaluation of the conglutinin titres, the 31 cattle in the first 2 categories that showed pronounced reactions, were grouped together. Likewise, the 29 animals that showed mild reactions or no reactions at all, were considered together. The conglutinin levels were inversely proportional to the severity of the reactions shown by the animals. This relationship was highly significant ($\chi^2 = 32{,}44$; $P \approx 10^{-9}$).

Thus, it can be seen in Table 1 that 26 cattle in the first 2 categories (those that showed pronounced reactions), had conglutinin titres in a lower range of 1:10 – 1:160 and 5 in the higher range of 1:320 and higher. Only 3 out of 29 animals in the last 2 categories (those that showed a mild febrile reaction or no reaction at all) had lower range titres and the majority (26) titres of 1:320 or higher. Furthermore, all 9 animals in category 1 had titres of 1:80 or lower and none of the 11 animals that failed to react had conglutinin titres lower than 1:160.

The titres of 10 sheep that had developed pronounced reactions of equal intensity were 1:80 and lower (Table 1).

It can be seen from Table 2 that there was a 4-fold decrease in the conglutinin titres of 2 of the 3 cattle that reacted to a second inoculation of *C. ruminantium* infected blood and a 8-fold decrease in that of the third animal.

* Rabbit haemolytic serum for sheep red cells, Wellcome Reagents, Ltd. Beckenham, England.

** Complement-fixation test diluent tablets, Oxoid Ltd., London.

Table 1: SEVERITY OF REACTIONS TO *C. RUMINANTIUM* IN RELATION TO CONGLUTININ TITRES

	Severity of reaction	Number of animals	Average duration of febrile reaction in days	Average incubation period in days	Average maximum temperature °C	Conglutinin titres			
						1:10–80	1:160	1:320	1:640 and higher
Cattle	High fever and other clinical signs	9	6	14	41	9	0	0	0
	High fever	22	5,1	14,7	41	12	5	3	2
	Mild fever	18	2,4	15,4	40,3	1	0	6	11
	No signs	11	–	–	–	0	2	6	3
	Total	60				22	7	15	16
Sheep	Severe reactions	10	5,2	9,4	41,9	10	0	0	0

Table 2: RECIPROCALS OF CONGLUTININ TITRES AT THE TIME WHEN THE INOCULATIONS WERE GIVEN, OF 3 CATTLE THAT REACTED TO A SECOND INOCULATION ONLY

Bovine No.	Inoculation 1	Inoculation 2
1	640	160
2	320	80
3	320	40

Table 3: DURATION OF IMMUNITY IN CATTLE: NUMBER OF ANIMALS THAT REACTED PER NUMBER CHALLENGED

Reaction at artificial immunization	Number of animals	Months after artificial *immunization*				
		3	6	12	24	Total
High fever and other clinical signs	9	0/2	0/2	0/3	0/2	0/9
High fever	25	0/6	0/6	0/6	1/7	1/25
Mild fever	18	0/5	0/4	0/5	1/4	1/18
No signs	8[1]	0/2	0/2	0/2	0/2	0/8
Total	60	0/15	0/15	0/15	2/15	2/60
Controls	20	3/5	5/5	3/5	3/5	14/20
Sheep inoculations		0/3	0/3	0/3	1/3	1/12

[1] 3 animals that reacted to second inoculation omitted and included under "High fever"

Duration of immunity: Reactions to challenge at intervals

None of the cattle in the 4 categories challenged at 3, 6 and 12 months following primary immunization showed any reaction at all (Table 3). The number of animals challenged in categories 1 and 4 on each occasion were admittedly too small to draw definite conclusions.

Of the remaining 15 animals challenged 24 months after primary immunization, 2 showed mild febrile reactions lasting 3 days in both cases following incubation periods of 11 and 14 days respectively. The peak temperatures were 40 and 40,2 respectively.

Five control animals were challenged together with the immunized cattle at each interval and 14 out of the 20 reacted.

C. ruminantium in the circulation of challenged immune cattle

In an attempt to establish whether *C. ruminantium* was circulating in the blood of animals following challenge, blood was collected on the 15th and 18th days from one animal in each of categories 1, 3 and 4 at each challenge interval and injected into sheep. None of the sheep which received blood of cattle challenged at 3, 6 and 12 months reacted and were subsequently shown to be fully susceptible. At the 24 month challenge one sheep which received blood from a reactor showed a mild febrile response and was subsequently only partially immune to heartwater. Blood from the other reactor and a non-reactor failed to infect sheep.

Monitoring of *C. ruminantium* infection in ticks

During the course of the experiment over 2 years, 12 adult *A. hebraeum* males were found on the experimental animals (Table 4). A sheep inoculated with 2 ticks collected 21 months after the commencement of the experiment, developed clinical disease and died. At autopsy the brain smear was found to be positive for heartwater. The other 7 sheep on which ticks were fed or which were inoculated with tick suspensions did not react and were fully susceptible when challenged with Ball 3 blood.

Table 4: ADULT *A. HEBRAEUM* MALES COLLECTED

Months after artificial immunization	Number of adult males	Sheep infected	Reaction in sheep
6	2	F/1/1[1]	–
10	3	F/1/2	–
12	1	F/1/0	–
21	2	I/1[2]	10/5/42
22	2	I/2	–
24	2	I/2	–

[1] F/1/1 = Both ticks allowed to feed on one sheep; one attached.
[2] I/1 = Both ticks inoculated into one sheep.

Intercurrent infections

Throughout the experiment blood smears prepared from animals during the febrile reaction were negative for *Babesia* and *Anaplasma*.

DISCUSSION

Statistical support of the relationship between levels of conglutinin in the serum of cattle and the severity of the reactions which they showed when they were immunized against heartwater, suggest that serum levels of conglutinin may play a role in the natural resistance of cattle to *C. ruminantium*. Although the conglutinin titres of the 60 cattle cannot be correlated with the severity of the reactions which they developed in such a way that there were no exceptions, a distinct pattern is nevertheless evident. Low conglutinin levels in 83 % of the animals that showed pronounced reactions stand in contrast to the high levels of the same percentage of cattle that either did not react or that showed mild febrile reactions. Of equal significance is the fact that all 9 animals that had developed anorexia and depression in addition to a high fever in spite of treatment, had conglutinin titres of 1:80 and lower, whereas not one of the cattle that had failed to react altogether and only one that had developed a mild febrile reaction, had low level titres.

Reduced conglutinin levels in the serum of 3 animals that reacted only after a second inoculation and the low range titres recorded in the sera of heartwater susceptible sheep, support this conclusion. Pronounced febrile reactions shown by the 3 cattle to the second inoculation correspond to the drop in conglutinin levels recorded at that time.

Low levels of conglutinin measured in the serum of 10 sheep that reacted uniformly and with pronounced febrile reactions to Ball 3 blood are consistent with the experience of research workers and practitioners in general that, with the possible exception of the Persian, sheep are highly susceptible to heartwater.

Under the present experimental circumstances, however, a final conclusion that serum conglutinin levels influence the susceptibility of cattle to heartwater, cannot be drawn, because of the lapse of time between the collection of the serum samples and their testing. It is known that conglutinin activity is lost after prolonged storage at –20°C[9]. Although the detrimental effect of storage on conglutinin must certainly be taken into account in the interpretation of the present findings, it cannot substantially alter the relationship between conglutinin titres and susceptibility to heartwater, because the sera were tested as a whole after having been stored for the same period of time.

The conglutinin titres which rarely exceeded 1:1280, were comparable with those reported for Friesland cows[6], but lower than those recorded in adult Zebu cattle[7]. Although *Bos indicus*-cross animals were used in this study, the lower titres can possibly be explained by the storage effect.

The possibility, a small one since very small numbers of ticks were collected over the course of 2 years, that some of the cattle had a tick-transmitted immunity when they were immunized, because they were maintained in a potentially enzootic heartwater area, must be taken into account. Moreover, the fact that 70 % of the control animals infected during the course of the experiment showed a degree of susceptibility similar to that of the immunized group, suggests that tick transmitted immunity in all probability did not contribute to the resistance of the animals that did not react or that had mild reactions.

The chief aim of this study was to determine the duration of immunity to *C. ruminantium* in cattle in the absence of infected ticks capable of enhancing the acquired immunity during the interval between the artificial immunization and the challenge of the animals. In view of the very small number of ticks recovered and the expected number of reactors among the controls, the possibility of tick-mediated enhancement of the acquired immunity can almost entirely be eliminated. At 3, 6 and 12 months after immunization, all 45 animals challenged were found to be solidly immune. After an interval of 2 years, 13 out of 15 animals were fully resistant to challenge, while the other 2 only reacted mildly to the challenge. It can therefore be concluded that cattle retain an artificially acquired immunity to heartwater unassisted by tick reinfection for at least 2 years.

This conclusion is reached with caution, though, because of the small number of animals challenged after the 24 month interval. Furthermore, should it be confirmed that conglutinin enhances the natural resistance of cattle to the heartwater agent, it is clear that serum levels of this substance should be taken into account to determine whether resistance to challenge of artificially immunized cattle can be ascribed solely to an acquired specific immunity and not perhaps to high levels of conglutinin. Moreover, there are factors which influence the conglutinin level. It varies according to the season[6], the breed of cattle[7], the nutritional state of the animal[6] and falls at parturition[5]. These factors may, therefore, influence the occurrence of heartwater in animals without an adequate acquired immunity.

It is therefore important that these observations on the duration of immunity to heartwater in cattle be confirmed, since the view is often expressed by veterinarians and stock owners that cattle on a given farm with low tick populations, because of intensive dipping, lose their immunity to heartwater because it is dependent on tick-mediated reinfection. The observation in this study that at least 13 animals challenged 2 years after immunization retained a solid immunity in the absence of reinfection points to the necessity of studies either to confirm or reject the above observation. Clarity on this matter is of cardinal importance in the epidemiology of heartwater.

Sheep were inoculated with blood drawn from the immunized cattle at the time when they were expected to react to the challenge blood to ascertain whether *C. ruminantium* was present in their peripheral blood as a result of the reinfection. With the exception of one sheep that showed only a mild febrile reaction for one day after having been inoculated with blood from a bovine that had developed a feeble reaction to challenge 2 years after immunization, none of the other 11 sheep reacted. This finding is inconsistent with the experience of Neitz *et al*[14], who found that *C. ruminantium* in the peripheral circulation of reinfected immune sheep induced fatal disease in susceptible sheep irrespective of whether a reaction in the former was demonstrable or not.

Neitz *et al*[14] pointed out that their experiment was limited to sheep, but nevertheless based their explanation of a particular phenomenon in the epizootiology of heartwater on it. They rightly pointed out that it has always been difficult to explain the continued presence of the heartwater agent in a high percentage of ticks on any given farm on the assumption that *A. hebraeum* larvae and nymphae become infected only by feeding on a reacting animal or for a short period after recov-

ery. To these workers the presence of *C. ruminantium* in the circulation of reinfected immune sheep implied that the agent in the blood of immune cattle or sheep reinfected through ticks can infect uninfected larvae and nymphae and thus maintain the infection on a farm.

In the present study blood from only 12 reinfected immune cattle was inoculated into sheep. This is a limited number but Neitz *et al*[14] based their view on results obtained on 11 immune sheep only. The present results therefore suggest that the above explanation of the maintenance of *C. ruminantium* on a given farm should be reconsidered.

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BOVINE BABESIOSIS: STEPS TOWARDS AN IRRADIATED VACCINE

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ABSTRACT: Purnell R.E.; Lewis D.; Brocklesby D.W.; Taylor S.M. **Bovine babesiosis: Steps towards an irradiated vaccine.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 339-344 (En) Agricultural Research Council, I.R.A.D., Compton, Newbury, Berks. U.K.

A series of experiments leading towards the field trial of an irradiated blood-derived vaccine against *Babesia divergens*, common cause of redwater in cattle in Europe, is described. Initially a number of isolates of *B. divergens* were made from the blood of sick animals in a variety of localities in the British Isles. These isolates were cryopreserved and then characterised by inoculation into groups of spenectomised Friesian calves, whose reactions were statistically analysed. Attempts were made to prepare a vaccine against *B. divergens* infection using diluted infected blood, but when these failed it was found that irradiation of infected blood within the range of 24 to 32 kilorads and its intravenous inoculation into calves produced the required immune response without pathogenic effects. An irradiated blood-derived vaccine produced by the irradiation of infected blood at 25 or 30 kilorads was used in a field trial in Ireland, and vaccinated calves were protected against a field challenge which caused redwater in 10 control cattle, six of which had severe reactions.

INTRODUCTION

Redwater in British cattle is caused by infection with *Babesia divergens*, a piroplasm very similar in morphology to *Babesia bovis*, and distinguished from it mainly by its choice of vector. *B. divergens* is transmitted solely by the tick *Ixodes ricinus* whereas *B. bovis*, a parasite of cattle in warmer parts of the world, is transmitted chiefly by ticks of the genus *Boophilus*. Although redwater is not a major cause of death in British cattle, it has a considerable nuisance value since fattening cattle in Ireland and the western parts of the British Isles are often affected by the disease, need treatment, and fail to achieve their optimal weight gain. The disease also restricts cattle movement within Britain since farmers in areas free of redwater are fearful of importing cattle from redwater areas, and conversely, farmers in redwater areas are reluctant to buy-in susceptible cattle from non-redwater areas.

We have developed a research programme around the possibility of finding a vaccine for protection of cattle against *B. divergens*, and this paper describes the sequence of stages in the programme.

ISOLATION AND CRYOPRESERVATION

The distribution of the tick *Ixodes ricinus* in Britain has been delineated by Barnett[1] (Fig. 1), although Ireland, where the tick is virtually ubiquitous, was excluded from the figure illustrating the distribution. We began by contacting veterinarians in practice throughout the distribution range of the tick, and asking them if they could provide us with blood samples from animals with redwater before therapy began. Veterinarians willing to cooperate were sent a simple kit consisting of a small cardboard box lined with 25 mm thick polystyrene and containing a medical flat bottle primed with heparin to prevent the blood clotting. The veterinarians were asked to collect 100 mℓ of blood from the sick animal into the bottle, pack it in the box with a blastic bag containing ice cubes, and post it to us. In this way we received 13 isolates from redwater cases in Great Britain, which are correlated with *I. ricinus* distribution (stippled area) on the map (Fig. 1). Isolations were also made from 2 cases in Northern Ireland and 2 in the Republic of Ireland.

When the blood reached Compton it was divided into two portions. One portion was mixed with an equal volume of phosphate-buffered saline containing 20 per cent dimethyl sulphoxide to prevent damage on freez-

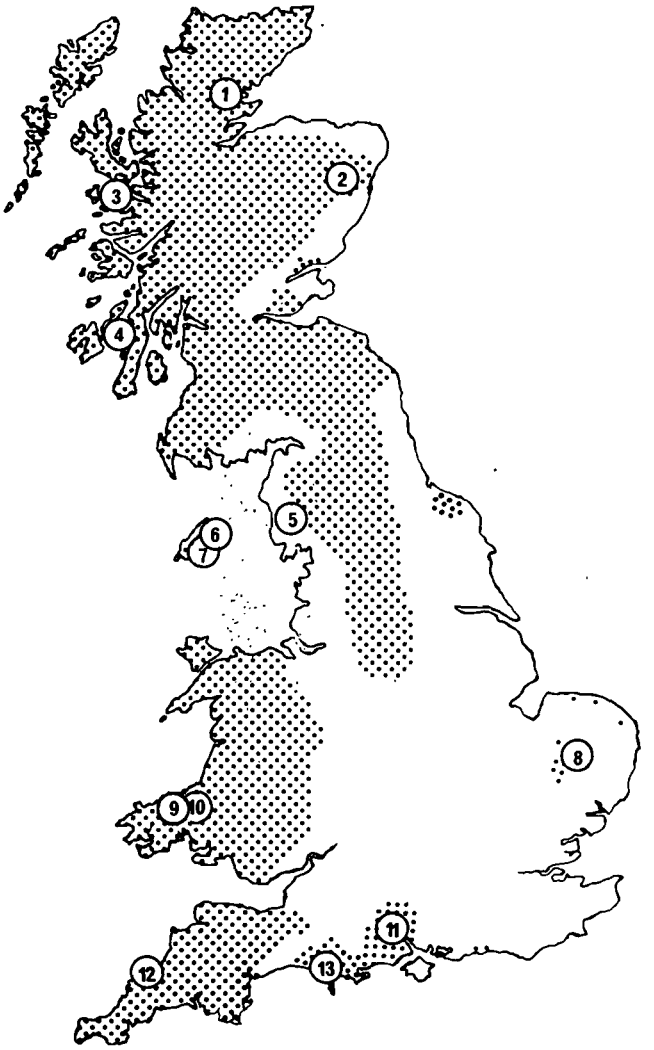

Fig. 1. Distribution of *Ixodes ricinus* in Britain correlated with *Babesia* strain collection sites.

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ing, and then dispensed into 5 mℓ aliquots in polythene vials, as described by Purnell et al (1975)[9]. The other portion was inoculated subcutaneously into a splenectomised Friesian calf, whose reaction was monitored by daily examination of Giemsa-stained thin blood smears and by daily recording of its rectal temperature. When *B. divergens* parasites were detected in its erythrocytes, 100 mℓ of blood was taken from its jugular vein. Three further splenectomised calves were each inoculated intravenously (i/v) with 1 mℓ of this blood and their reactions, including changes in haematological values, were monitored and recorded. The remainder of the blood was deep-frozen as a second stabilate. In this way, two stabilates were made of each field strain of the parasite, and the strain was also characterised in splenectomised Friesian calves.

CHARACTERISATION

Observations were made for 30 days on the groups of 3 splenectomised calves which had each received 1 mℓ of blood from the animal receiving the field blood. After preliminary examination of the data obtained, five measured variables were selected as indicating aspects of the severity of response of the calves. These were maximum parasitaemia, maximum febrile response, total febrile response in excess of 39,4°C, highest mean corpuscular volume (MCV) and lowest packed cell volume (PCV). A principal component analysis was then carried out using this data[6]. The results of that analysis for 5 of the field strains isolated are shown in Figure 2.

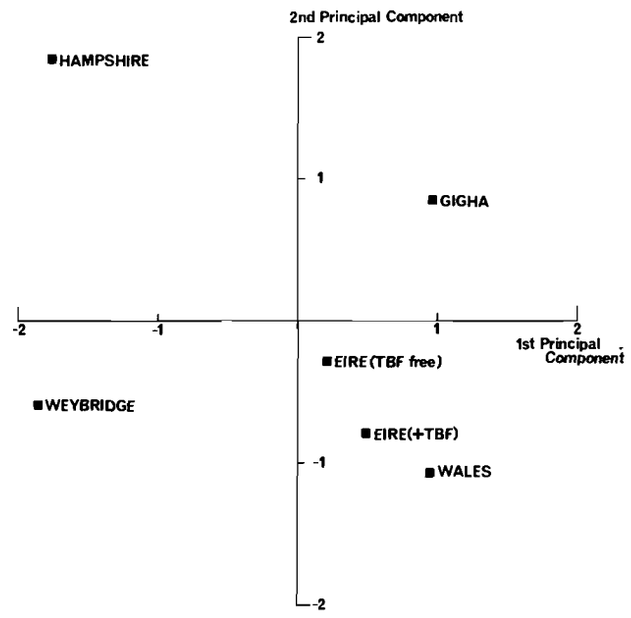

Fig. 2. Principal component analysis of pathogenic characteristics of 5 *Babesia divergens* isolates in splenectomised Friesian calves.

The first principal component consisted largely of four variables (maximum parasitaemia, maximum febrile response, highest MCV and lowest PCV) while the second consisted largely of two variables (maximum febrile response and total febrile response above 39,4°C). The individual scores for each principal component were calculated for each animal, and the scatter diagram (Figure 2) is plotted using the average score for each isolate.

The analysis suggested that there were different characteristics of the different isolates; in fact, the isolates had individual pathogenic identities when inoculated into splenectomised Friesian calves. The results of the two characterisations of the Republic of Ireland isolate lend support to this concept. The isolate was originally contaminated with *Ehrlichia phagocytophila*, the agent of tick-borne fever (TBF) which was removed by selective chemotherapy. The characteristics of the isolate were checked again after removal of the contaminant, and the new point for this isolate on the scatter diagram was seen to be nearly coincident with the original point. This indicates two things (a) TBF did not interfere markedly with the pathogenicity of *B. divergens* in this case and (b) the results of the analysis are reproducible.

There appeared to be some geographical significance in the pattern obtained in the scatter diagram in that the Welsh and Irish isolates appeared to produce similar responses in inoculated calves and the Scottish isolate produced responses similar to those two in the dimension of the first principal component. The disparity in the results obtained by inoculating the Weybridge strain and the Hampshire isolate into groups of calves was of particular interest to us since the veterinarian in Hampshire deliberately selected an animal from the exact area of origin of the Weybridge strain, isolated 25 years previously by his father. It was tempting to speculate that the demonstrated difference was due to continued syringe passage of the Weybridge strain in the laboratory.

TITRATION

The basis of the redwater vaccines used extensively in Australia, South Africa and in certain Central and South American countries is the dilution of blood obtained from artificially-infected donor calves. The success of these vaccines depends on the fact that animals inoculated with low numbers of viable *Babesia* parasites have mild reactions and are subsequently resistant to field challenge. Regrettably this is not always the case, animals receiving the vaccine sometimes have untoward reactions which require chemotherapeutic restraint whereas in other instances the vaccine fails to give sufficient protection[5].

Having established that isolates of *B. divergens* have individual pathogenic identities in splenectomised Friesian calves, we decided to titrate the numbers of parasites inoculated to see if increasing dilution changed these pathogenic identities so that the animals would have mild reactions but be immune to subsequent homologous challenge. And thus, we attempted to produce a diluted blood vaccine.

Groups of three splenectomised calves were inoculated with infected blood from a calf which had been inoculated with a stabilate of the Republic of Ireland 2 isolate of *B. divergens*. The blood was diluted in phosphate-buffered saline (PBS, pH 7,2) to give inocula from 10^9 down to 10^5 parasites. Increasing dilution of the blood resulted in extended prepatent periods before parasites were detected in blood smears and until the animals had febrile responses. When their reactions did begin, however, they were all characteristic of the

isolate. A second experiment was carried out in which lower numbers of parasites were inoculated and here there appeared to be a 'cut off' point at a dose of 10^3 parasites. At this dose only 2 of the 3 animals reacted, and the remaining animal was fully susceptible to homologous challenge whereas animals receiving all the other dilutions had been immune to challenge.

Titration was clearly not a suitable way to alter the pathogenic identity of the isolate, and so we investigated a number of other ways to alter the host-parasite equilibrium in favour of the host. Inoculation of *Mycobacterium bovis* strain BCG had given very encouraging results in mice, where non-specific immunity had been shown to protect the mice against *B. microti* and *B. rodhaini*[4], but we were unable to protect our cattle against *B. divergens* infection by this means, even when massive doses of BCG were used[2]. Again, Todorovic et al (1973)[11] have shown that a series of intra-muscular inoculations of cattle with lyophilised plasma obtained from animals reacting to infection with *B. bovis* and *B. bigemina* will protect the inoculated cattle against infection in the field. Using our experimental system we found that we could only obtain a limited protection against infection by three successive intramuscular inoculations of lyophilised plasma + adjuvant[8]. We decided, therefore, to try to attenuate the parasite by irradiation.

IRRADIATION

Phillips (1970)[7] reported that the inoculation of irradiated *B. rodhaini* parasites into mice and rats conferred some protection against subsequent homologous challenge, and suggested that the possibility of extending his experiments to economically important piroplasms of cattle merited investigation. We carried out a small experiment on *B. major* in splenectomised calves[3] and the results obtained suggested that the irradiation of parasitised erythrocytes at doses in the region of 30 krads might result in attenuation of the parasites without loss of immunogenicity.

In our first *B. divergens* experiment, groups of 3 splenectomised calves were inoculated with $1{,}2 \times 10^{10}$ *B. divergens*-infected erythrocytes obtained from a donor calf infected with a Republic of Ireland 2 isolate. The erythrocytes had been irradiated at 24, 28, 32, 36 or 40 krads using a ^{60}Co source. Two control groups of calves were inoculated with $1{,}2 \times 10^7$ or $1{,}2 \times 10^4$ non-irradiated parasites so that the linear relationship between dose and prepatent period could be established. Any increase in prepatent period in animals receiving irradiated blood without alteration of the pathogenic identity of the parasite could thus be put down simply to a killing effect of the irradiation. The results of the experiment are summarised in Table 1.

All the animals receiving blood irradiated at 24, 28 and 32 krads had mild reactions and all but one were immune to challenge. One animal in the 32 krad group had a mild reaction on challenge. Animals receiving blood irradiated at 36 and 40 krads had a limited and variable protection against challenge. Animals in the control groups all had severe reactions, and their prepatent periods were dose related as seen in the titration experiments. In contrast, not only were prepatent periods extended in animals receiving irradiated parasites, but also the reactions of the calves were much milder than normal.

These results encouraged us to carry out a second experiment in which groups of 3 intact Friesian calves were inoculated with $5{,}9 \times 10^9$ *B. divergens*-infected erythrocytes obtained from a donor calf infected with the Republic of Ireland 2 isolate. The same irradiation doses were used, but in this case challenge was with an heterologous isolate of *B. divergens* recently isolated from the field (Ulster 3). The results of this experiment are summarised in Table 2.

In this experiment the reactions of the calves were much milder than when splenectomised calves were used, since blood passage of *B. divergens* in intact animals does not induce reactions of corresponding severity to natural tick-induced reactions. Interpretation of

Table 1: REACTIONS OF GROUPS OF 3 SPLENECTOMISED CALVES TO THE INOCULATION OF IRRADIATED *B. DIVERGENS*-INFECTED BLOOD AND TO SUBSEQUENT HOMOLOGOUS CHALLENGE

Number of parasites inoculated	Irradiation dose in kilorads	Parasitaemia		Febrile response		Summarised reaction to inoculation	Reaction to homologous challenge
		Prepatent period in days	Highest parasitaemia x 10^3/cu mm	Prepatent period in days	Highest fever in °C		
10^{10}	24	7,7	104	7,7	39,9	3 MR	3 NR
10^{10}	28	9,0	108	8,0	40,4	3 MR	3 NR
10^{10}	32	11,0	75	11,0(2/3)	40,1(2/3)	3 MR	1 MR 2 NR
10^{10}	36	10,0(2/3)	614(2/3)	9,3	40,8	1 SR 1 MR 1 NR	1 NR 1 MR 1 SR
10^{10}	40	–	–	11,0(1/3)	39,7(1/3)	3 NR	2 SR 1 MR
10^7	0	4,0	1041	4,3	40,7	3 SR	3 NR
10^4	0	6,0	1277	5,7	41,0	3 SR	3 NR

NR = no reaction
MR = mild reaction
SR = severe reaction

Table 2: REACTIONS OF GROUPS OF 3 INTACT CALVES TO THE INOCULATION OF IRRADIATED *B. DIVERGENS*-INFECTED BLOOD AND TO SUBSEQUENT CHALLENGE WITH A HETEROLOGOUS STRAIN OF *B. DIVERGENS*

Group	Irradiation dose in krads	Summarised reactions to inoculation	Summarised reactions to challenge
A	0	3 MR	3 VMR
B	24	1 VMR 2 NR	1 MR 2 VMR
C	28	1 VMR 2 NR	1 MR 1 VMR 1 NR
D	32	3 VMR	2 MR 1 VMR
E	36	2 VMR 1 NR	2 SR 1 VMR
F	40	1 VMR 1 NR	2 SR
G	Challenge control	–	2 SR 1 MR

the severity of reaction of intact animals depends on the examination of thick blood smears and on haematological effects, such as a drop in an infected animal's packed cell volume. Animals receiving irradiated blood had either very mild reactions or no overt reactions, while the control animals, which received the same number of non-irradiated parasites, all had mild reactions. On challenge, the animals which had received blood irradiated at 24, 28 or 32 krads or infected non-irradiated blood all had mild, very mild or no overt reaction. In contrast 4 of 5 surviving animals which had received blood irradiated at 36 or 40 krads and 2 of 3 challenge control animals had severe reactions, where parasitaemias in excess of 10^7 parasites/mℓ of blood were recorded.

These results confirmed those of the experiment on splenectomised calves and suggested that irradiation of infected blood at 24, 28 or 32 krads had resulted in an inoculum which would induce mild reactions in inoculated calves but would give protection against *B. divergens* challenge. The next stage was to set up a field trial in which animals protected by inoculation of irradiated blood would be exposed to severe tick-induced *B. divergens* challenge.

VACCINATION

In May 1978, Mr W Martin a veterinarian in practice at Lurgan, Co Armagh, Northern Ireland sent a redwater sample to Stormont from an infected bullock. The sample was passed to Compton, a stabilate made as described previously and the isolate designated Ulster 3. Subsequently negotiations took place with the local farmer, and the field grazed by the bullock together with four adjoining fields, was rented so that a field trial could take place from August until October 1978.

Thirty Friesian bull calves from a tick-free area were purchased, and maintained at the Veterinary Research Laboratories, Stormont, Northern Ireland. From June 1978, when they were six months of age, they were grazed at Stormont on tick-free pasture. At Compton in July 1978, an Ulster 3 stabilate was revived by passage through a splenectomised Friesian calf and subsequently infected blood was harvested from a further calf into which the *B. divergens* had been subinoculated. The infected blood was divided into two portions, one of which was irradiated at 25 krads and the other at 30 krads. The irradiated blood was flown immediately to Stormont where 10 calves were each inoculated with 20 mℓ aliquots of blood containing 10^{10} parasites irradiated at each dose level. The remaining 10 calves were to serve as uninfected controls for the field exposure. The inoculated calves were observed daily for three weeks and their reactions scored as follows:

– = negative
+ = 1 parasite seen in a blood smear
++ = several parasites seen
+++ = a parasitaemia between 1/1000 and 10/1000 observed
++++ = a parasitaemia of 10/1000 or above observed.

Four weeks after vaccination, all the calves were transported to the field trial site and released. They were then observed three times weekly,and serum collection for detection of *B. divergens* anti-bodies using the indirect fluorescent antibody test (IFA) continued on a weekly basis. In addition, ticks were collected from the pasture each week by a team of 4, using tick 'flags' for 20 minutes along defined areas of the hedged perimeters of the fields.

Observations on the calves continued during the 2 months' exposure at the field trial site and for a further month after they had returned to Stormont. The results of the examination of the animals are summarised in Table 3.

Table 3: AGHADAVEY FIELD TRIAL OF IRRADIATED *BABESIA DIVERGENS* BLOOD VACCINE: SUMMARISED REACTIONS OF GROUPS OF VACCINATED CALVES TO FIELD CHALLENGE WITH *B. DIVERGENS*

Group	Vaccination *Babesia* score	Field exposure *Babesia* score
1 25 Krad irradiated blood	2 +++ 1 ++ 3 + 4 –	1 +++ 2 ++ 2 + 5 –
2 30 Krad irradiated blood	3 +++ 2 + 5 –	4 +++ 4 ++ 2 –
3 Control		6 ++++ 1 +++ 2 ++ 1 +

The most striking effect of exposing the calves in the field was that all animals immediately became heavily infested with ticks and developed tick-borne fever (*Ehrlichia phagocytophila* infection) within one or two weeks. Although this result is not indicated in Table 3, the effect of this infection is illustrated in the significant decrease in the animals' packed cell volumes as shown in Figure 3.

As far as the *B. divergens* challenge was concerned all the susceptible control animals had demonstrable

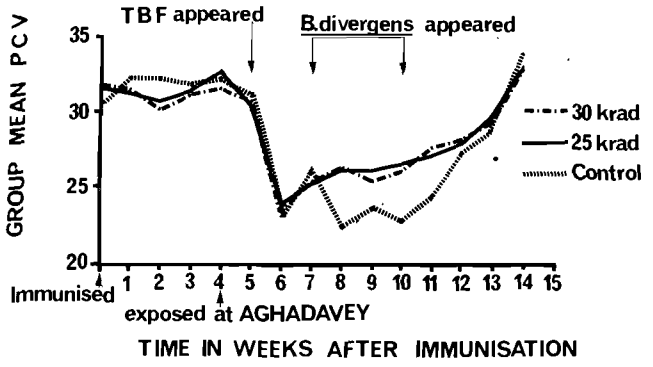

Fig. 3. Aghadavey Field Trial of irradiated *Babesia divergens* blood vaccine: Group mean packed cell volumes correlated with time after immunisation.

parasitaemias by day 43 of exposure. Six of them developed clinical babesiosis, and one barely survived its clinical episode. In contrast none of the calves which had previously received the irradiated infected blood had severe reactions and a comparison of the haematological data from the groups revealed that there were a number of significant effects of babesiosis in the control group. The group packed cell volumes throughout the period of the trial are shown in Figure 3.

The results of tick flagging are shown in Figure 4.

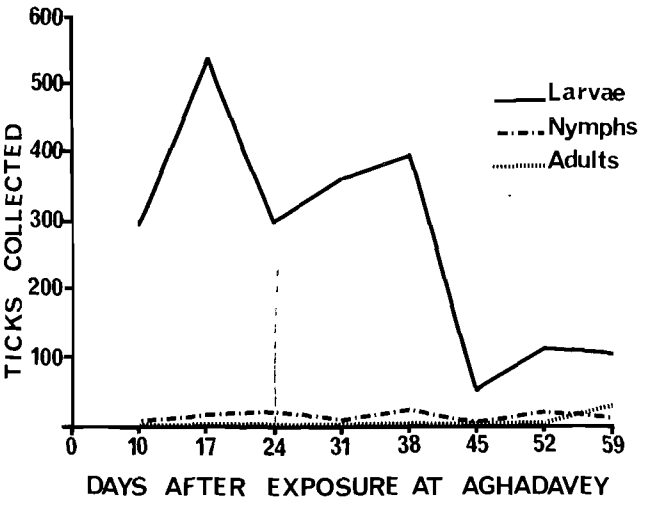

Fig. 4. Aghadavey Field Trial of irradiated *Babesia divergens* blood vaccine: *Ixodes ricinus* tick collection by flagging correlated with exposure of cattle.

The vast majority of ticks found by flagging were larvae, whose numbers were maintained at a high level from the end of August until the end of September, when they fell significantly. Nymphs stayed at a variable low level throughout the period of the trial, whereas adults were only detected at the last collection on October 13th.

The results of the IFA test are shown in Figure 5.

They show that there was an initial rise in group mean antibody titre in Groups 1 and 2 resulting from vaccination and a subsequent further increase in titre resulting from *B. divergens* challenge in the field.

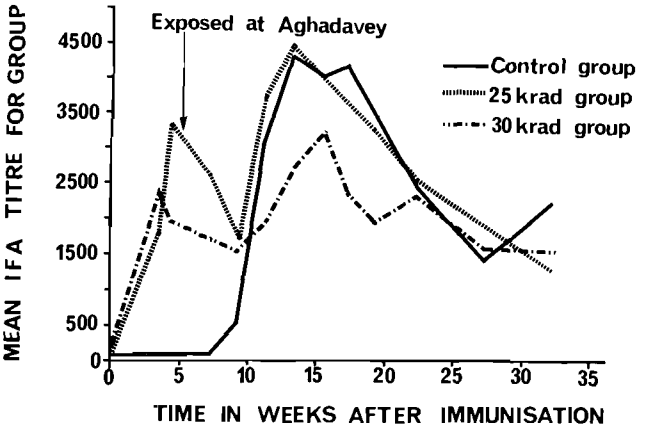

Fig. 5. Aghadavey Field Trial of irradiated *Babesia divergens* blood vaccine: Group mean I.F.A. titre correlated with time after immunisation.

The results of the field trial, where animals were exposed to unlimited tick challenge with an homologous strain of *B. divergens,* demonstrated that the method of vaccination was satisfactory. No severe reactions to vaccination occurred and the vaccinated animals withstood a field challenge which resulted in clinical babesiosis in 6 out of 10 control animals exposed with them.

These results have been reported more fully elsewhere[10] and were briefly referred to in a magazine called "What's new in farming" and resulted in a number of enquiries to the editor for further information. The point of origin of each of these enquiries was plotted and superimposed on the '*I. ricinus* in Britain' distribution map. The results are shown in Figure 6.

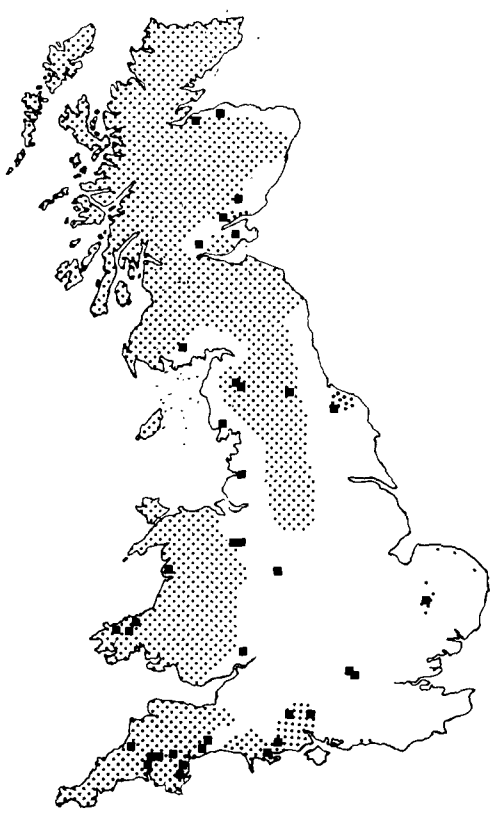

Fig. 6. Aghadavey field trial of irradiated *Babesia divergens* blood vaccine: Enquiries from farmers correlated with distribution of *Ixodes ricinus.*

There was clearly a considerable interest in the prospect of a redwater vaccine for Britain, and we hope to carry out further field trials of this, and improved, vaccines.

ACKNOWLEDGEMENTS

The authors are most grateful to Mrs A. Brabazon for technical assistance at Compton and to Messrs McCann, Mallon and Green and Mrs A. Strain for technical assistance at Stormont. They are also grateful to Mr P. Adams for irradiating the blood samples, to Mrs B. Kitchenham for carrying out the statistical analysis, to Mr D. Davies and Dr D. Baggott for splenectomising the calves and to Mr W. Martin, veterinarian-in-practice at Lurgan for his help in arranging the field trial.

The work described in this paper forms part of a coordinated programme of research on tick-borne diseases of livestock under the auspices of the International Atomic Energy Agency. We are grateful to the United Kingdom Overseas Development Administration for financial support to one of us (DL) (Research Project R3270).

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IN VITRO INFECTION AND TRANSFORMATION OF LYMPHOID CELLS BY SPOROZOITES OF *THEILERIA PARVA* AND *T. ANNULATA*

C.G.D. BROWN

ABSTRACT: Brown C.G.D. ***In vitro* infection and transformation of lymphoid cells by sporozoites of *Theileria parva* and *T. annulata*.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 345 (En). Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin EH25 9RG Scotland.

This paper describes a method developed for the establishment of bovine lymphoblastoid cell lines infected and transformed by *Theileria parva* or *T. annulata* and the application of this technique to the study of the life cycle and the mechanism of immunity in bovine theilerioses.

Cultures of bovine lymphoid cells are established from peripheral blood or tissues of the lymphoid organ, seeded over fibroblastic feeder layers and incubated in medium designed primarily for human lymphoid cell lines. Suspensions of *Theileria* sporozoites are harvested from pre-fed infected ticks which are surface-sterilized and ground in medium containing bovine plasma albumin, antibiotics and fungistat. This suspension is centrifuged at low speed and the supernatant filtered through membranes of pore size selected to exclude contaminants but permit passage of the majority of sporozoites. This filtrate can then be inoculated into the lymphoid cultures. Cultures infected in this manner may exhibit the first intracellular forms of the parasite within 24 hours, macroschizonts on the second day, and can be subcultured as early as the fourth day after infection. Blastoid transformation is observed in these cultures and it is evident that this transformation is parasite-associated, the presence of the organism within a cell being a prerequisite to its continued multiplication. The division of macroschizonts and their host cells is interdependent and parasitized cells divide to give rise to daughter cells which are also parasitized. Once transformed, cell lines of *Theileria*-infected lymphoblasts can be maintained in exponential growth phase at cell concentrations of up to $2{,}5 \times 10^6$ mℓ^{-1}, with a population doubling time of 18–21 hours. Between 90–100 % of cells are parasitized, the schizont nuclear number remaining constant for more than 300 passages over 3 years.

Such cultures may be used to immunize cattle against tropical theileriosis (*T. annulata* infection) and East Coast fever (*T. parva* infection). They also provide antigens for immunological studies and serodiagnostic methods and offer a system for evaluating potential chemotherapeutic agents for the treatment of theileriosis. The novel technique of infection of bovine lymphocytes in cell cultures provides a system for evaluating the infectivity of fresh and cryopreserved suspensions of sporozoites and for examining humoral and cellular immune mechanisms *in vitro*.

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THERAPEUTIC IMPLICATIONS OF *BABESIA CANIS* INFECTION IN DOGS

D.J. MOORE

ABSTRACT: Moore D.J. **Therapeutic implications of *Babesia canis* infection in dogs.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 346-352 (En) Orange Grove Veterinary Hospital, 119 Louis Botha Ave, Orange Grove 2192, Johannesburg, Rep. of South Africa. The therapeutic regime used in the treatment of dogs with *Babesia canis* infection differed between dogs with mild (uncomplicated) and severe (complicated) disease. In addition to the treatment given to dogs with mild disease, the dogs with severe disease received intravenous fluids, heparin and blood transfusion together with other supportive measures. Mortalities occurred only in the group with severe disease and were attributed to disseminated intravascular coagulation.

INTRODUCTION

The therapeutic agents previously used in the treatment of canine babesiosis included powdered chloride of ammonia and extract of belladonna[22]; quinine, benzoate of soda and carbolic acid[23]; calomel and quinine[24] but has changed markedly with the discovery of the specific babecidal agents. In addition supportive procedures such as blood transfusion[7,40], fluid therapy[7,40], alkalinizing agents[7,40], liver protectants[40], diuretics[7], restricted exercise[52] and improved nutritional status[7,52] have been advocated. The specific babecidal agents presently available in the Republic of South Africa include:

Azo-naphthalene dyes

Trypan blue and trypan red were the first effective drugs against *Babesia canis*[48]. Trypan blue was more effective than trypan red and the approximate dosage rate used was 1 mℓ/kg body mass of a 1 % solution (10 mg/kg) given subcutaneously, although relapses sometimes occurred[48]. Henning[20] advocated the use of a freshly prepared, sterile 1 to 2 % solution administered slowly intravenously as subcutaneous administration resulted in abscess formation[20,48]. The dosage rate of trypan blue for use in the dog is variously stated as being 5 to 10 mℓ[56], or 5 to 25 mℓ[52] of a 1 to 2 % solution, 2 mg/kg body mass of a 1 % solution[6] or 20 to 150 mg of a 1 to 2 % solution[9]. The drug does not eliminate all parasites and recovered animals are premunized[35,48]. Trypan blue has the disadvantage of staining all the body tissues and secretions blue-green which can take several weeks to disappear[48]. The drug is known to block the reticulo-endothelial system and potentiate the generalized Shwartzman reaction[41,53], however, the dose used to achieve this blockade was approximately 300 mg/kg body mass[53].

The Acridine Dyes

Levine[35] cites Stephan & Esquiber[55] as introducing acriflavin in 1929. The dye does not eliminate all parasites, and recovered, treated animals are premunized[35]. The drug must be given intravenously due to its necrotoxic property[20]. The dosage rate in dogs is variously stated as 1 to 3 mℓ/kg body mass of a 0,1 to 2 % solution[35] or 0,6 mℓ/kg body mass of a 2 % solution[52]. Malherbe[39] cites Domagk & Kikuth[10] as finding the dose of 5 mg/kg as effective although a single dose of 15 mg/kg body mass, or 10 mg/kg body mass administered intravenously on two occasions a week apart, could produce "sterilization" of the infection.

The Quinoline Derivatives

Levine[35] cites Kikuth[27] as introducing Acaprin* in 1935. The quinoronium sulphate derivatives are more active against *B. canis* than trypan blue or acriflavin[25], but the therapeutic index is low[12,51] due to the stimulatory effect on the parasympathetic nervous system[12]. The quinoline derivatives possess an anticholinesterase action and stimulate histamine release which results in toxic signs such as salivation, urination, dyspnoea, cyanosis, apnoea, collapse and death[12]. These symptoms can be partially alleviated by atropine and 2-aldoxeine methiodide – a cholinesterase reactivator[12]. The recommended dosage rate of Babesan† is 0,25 mℓ/kg body mass of a 0,5 % solution given subcutaneously or 0,25 mg/kg body mass of a 0,5 % solution[20]. At this dosage rate recovered, treated animals are premunized although higher dosages can sterilize infections[25]. Eyre[12] determined that at a dosage of 1 mg/kg body mass toxic signs could be expected. Symptoms of intolerance consisting of restlessness, muscular spasms, salivation and defecation may occasionally be exhibited by treated animals at the recommended dosage[25]. The toxic signs can be minimized by dividing the therapeutic dose into 2 or 3 divided doses given a few hours apart[35].

The Aromatic Diamidines

Lourie and Yorke[37] introduced these compounds in 1939 and recommended the use of Phenamidine‡ at a dosage rate of 10 mg/kg body mass administered subcutaneously. Relapses occasionally occurred 6 to 33 days after the administration of a sub-curative dose. *B. canis* rapidly developed a resistance to the aromatic diamidines after the administration of sub-curative doses[15,37] and these resistant strains remained refractory to treatment with quinoronium sulphate[15]. Phenamidine‡ was found to be less toxic than Acaprin*[37], although doses of the diamidines which approach the toxic level cause hyperglycaemia and result in the depletion of liver glycogen[57]. A significant rise in blood urea and non protein nitrogen occurs while the serum calcium and potassium levels fall at dosages which do not affect the blood glucose concentration[58]. The diamidines have a depressant action on the circulatory system and cause a fall in blood pressure, due mainly to peripheral vasodilation, which is only partially accounted for by stimulation of the parasympathetic nervous system and which is antagonized by calcium[57]. The diamidines increase the blood histamine concentration due to the release of

* Bayer
† Imperial Chemical Industries
‡ Maybaker

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histamine from the tissues together with their inhibitory effect on histaminase, these actions may influence the toxicity of these compounds[4]. The therapeutic dosage rate of Phenamidine† is 0,3 mℓ/kg body mass of a 5 % solution administered subcutaneously[9] or 15 mg/kg body mass [46]. The therapeutic dose can cause local irritation at the site of injection as well as anaphylactoid reactions such as angioneurotic oedema, nausea, salivation, vomition and diarrhoea[9 31 46].

Malherbe[39] cites Bauer[2] as introducing Berenil* in 1955. Acute anaphylactoid reactions are encountered less frequently than with Phenamidine†[46]. Berenil* is rapidly excreted via the kidneys[21] although Naude[46] cites Launoy, Guillot & Jonchere[33] as reporting that no detoxification of the diamidines occurs in the body and that these compounds accumulate mainly in the liver, kidneys and brain. At low dosage the treated, recovered animal is premunized while high dosage results in sterilization of the infection[3 21]. The recommended therapeutic dose to premunize dogs is 3,5 mg/kg body mass of a 7 % solution administered either subcutaneously or intramuscularly[21], dosage of 4 to 5 mg/kg body mass will sterilize infections in some dogs[3] while dosage of 12 mg/kg body mass will sterilize infections in all instances[3]. Dosage of over 10 mg/kg body mass will cause toxic disturbances of the central nervous system (CNS)[3] and it is not recommended to exceed a dose of 7 mg/kg body mass in the dog[21]. In rare instances even therapeutic doses given to dogs can cause toxic disturbances of the CNS and these effects are attributable to hypersensitivity of the individual animal[5 21]. The prepared Berenil solution should be kept in sealed glass vessels and protected from direct sunlight[21]. In such containers the solution retains stability for 14 days in a cool place, 5 days at 20°C (68°F) and 3 days at 30°C (86°F)[21].

The toxic properties of the diamidines have been reported as CNS derangements[5 18 31 36 46 49] which include behavioural changes, nystagmus, ataxia, extensor rigidity, opisthotonus, coma and death. Toxic symptoms such as haemorrhagic gastroenteritis, hepatic failure and muscular degeneration have been reported[31 32 46 47].

It is the purpose of this paper to illustrate the importance of a multifaceted therapeutic approach if the critically ill patient with biliary fever is to be salvaged.

MATERIALS AND METHODS

Twelve randomly selected naturally infected clinical cases of canine babesiosis which were presented for diagnosis and treatment were studied. The clinical symptoms, urinary findings and categorization of the patients has been previously reported[43 44].

The haematological and coagulation findings have been previously discussed[43 44] and are documented in Table 1.

The method of treatment varied substantially between the two categories of infected dogs. The mild (uncomplicated) patients were treated as outpatients while the severe (complicated) patients were hospitalized for intensive therapy. Each patient was accurately weighed during the course of the clinical examination. Observations were made as to the degree of dehydration, if present, utilizing the criteria of Finco[13].

† Maybaker
* Hoechst

Mild Category

The therapy employed on the day of presentation, once a definitive diagnosis had been established and the patient categorized, was similar in every instance. Each animal received a babecide in the form of Berenil* at the dosage rate of 3,5 mg/kg body mass of a 7 % solution and given subcutaneously. Each animal also received procain penicillin** at the dosage rate of 10,000 u/kg body mass, together with Prednisolone† at the dosage rate of 1 mg/kg body mass and Vitamin B Complex‡ at the dosage rate of 0,25 mℓ/5 kg body mass all given subcutaneously. The owner was instructed to return the dog for a second examination 12 to 24 hours after the initial examination.

In each instance the response to therapy was excellent as adjudged by the return of appetite, improvement in habitus, return of the body temperature to normal and a negative capillary blood smear for *B. canis* parasites.

The treatment utilized on the day following initial presentation was identical to the first except that no babecide was administered. The owners were instructed to supplement the conventional ration with a high biologic value protein diet using egg and liver, to provide free access to drinking water and to restrict exercise for one week.

Severe Category

The therapeutic regime employed was similar in each instance and can be summarized as follows. A 14 to 16 gauge cephalic or jugular catheter§ was immediately placed according to standard technique[28].

The patient was rapidly infused with a balanced polyionic solution[a] at the dosage rate of 40 mℓ/kg body mass to which was added 8,5 % sodium bicarbonate[b] at the dosage rate of 3 milli-equivalents/kg body mass. An intravenous bolus of heparin[c] was infused in all except Case 7 at the rate of 100 units/kg body mass. A sterile freshly prepared solution of 1,5 % Trypan blue[d] was added to the polyionic solution at the dosage rate of 0,5 mℓ/kg body mass (7,5 mg/kg). Betamethasone[e] was infused as an intravenous bolus at the rate of 0,1 mg/kg body mass.

The fluid infusion was followed by a fresh whole blood transfusion in Cases 8, 9 and 10 and the volume of blood calculated using the following formula[40]:-

$$\frac{\text{Packed cell volume of donor} - \text{Packed cell volume of recipient}}{\text{Packed cell volume of donor}} \times \frac{80}{1} = \begin{matrix}\text{millilitres of blood per kilogram body live mass of recipient.}\end{matrix}$$

After completion of the blood transfusion or initial fluid infusion (Cases 7, 11 and 12), fluid infusion was continued using Plasmalyte B[f] or 5 % Dextrose solution* at a rate which varied from 10 to 40 mℓ/kg body

* Hoechst;
** Pfizer;
† Deltacortril-Pfizer;
‡ Centaur;
§ Venocath-Abbot
[a] Plasmalyte B - Baxter;
[b] Saphar;
[c] Pulvarin - Allen & Hanburys;
[d] Centaur;
[e] Betsolan soluble - Glaxo;
[f] Baxter

Table 1: HAEMATOLOGICAL AND COAGULATION FINDINGS

Case No.	1[e]		2[e]		3[d]	4[d]		5[d]		6[e]		7[e]	8[e]		9[e]		10[e]			11[d]			12[d]		Normal Range
Day	1	2	1	2	1	1	2	1	2	1	3	1	1	5	1	3	1	2	4	1	3	6	1	2	
PT patient	6,4	6	8	6	8	9		8,4		7,25		7	7		6		12	10	9,9	9,6	9,5	10,3	9,2		
Control	6	6	6	6	7,4	6,8		8		6		7	7		6		6	6	9,2	9	9	9	8,5		6–14 sec.[a]
APTT patient	45	32	31	33	21,6	27,5		20		27		38	41	38	28		40	47	29	25,2	24,2	28	34,5		
Control	25	25	33	33	15,1	15,4		16,4		25		25	22	25	25		25	22	24	14,2	14,2	15	16,8		15–25 sec.[b]
Fib	5,28		5,45	4,8	1,23	5,44		7,31		2,03		5,28	4,35	9,5	6,3					6,7	5,4	4,62	6,2		1–5 g/l[a]
FDP	<8		<8	<8	<10	<10		<10		<8		20	>40	10	25	<8	>40	40	<8	>40	<10		<10		<10 mg/l[c]
Plat.	25		16	110	23	31	35	25	80	33	57	10	14	27	23	71	4,4	71	110	14	17	25	30	92	200–500 x 10^9/l[a]
WCC	18,8		5,2	12	15	5,7	15,3	4,9	12	14,3	7,5	3,8	10,7	22,5	6	24	15,1	28,6	30,5	11,6	15	13	13,4	22,8	6 – 17 x 10^9/l[a]
RCC	3,11		6,5	5,8	3,95	4,55	4	6,4		2,4	3,6	7,6	3,4	6,4	4,5	4,9	1	4,3	4,1	6	4	4,6			5,5 – 8,5 x 10^{12}/l[a]
Hb	74		168	146	101	110	102	161		74	90	187	82	164	108	130	27	105	99	120	78	83		187	120–180 g/l[a]
PCV	0,23	0,26	0,48	0,43	0,28	0,31	0,3	0,45	0,44	0,2	0,29	0,52	0,27	0,46	0,31	0,36	0,09	0,32	0,32	0,33	0,23	0,27	0,63	0,52	0,37 – 0,55[a]
Neut	0,89		0,6	0,69	0,61	0,74	0,64	0,9	0,74	0,64	0,71	0,62	0,67	0,88	0,68	0,79	0,76	0,81	0,78	0,8	0,8		0,82	0,8	0,67 – 0,77[a]
Mono	0,03		0,03	0,07	0,11	0,04	0,06	0,02	0,05	0,13	0,13	0,05	0,03	0,02	0,04	0,05	0,08	0,06	0,03	0,05	0,12		0,07	0,09	0,03 – 0,1[a]
Lympho	0,08		0,37	0,24	0,28	0,22	0,3	0,08	0,21	0,2	0,16	0,33	0,3	0,1	0,28	0,16	0,16	0,13	0,19	0,15	0,08		0,1	0,1	0,12 – 0,3[a]
Eosino										0,03		0,01											0,01	0,01	0,02 – 0,1[a]

[a] Normal ranges from Schalm O W, Jain N C, Carrol E J 1975

[b] Normal range from Moore D J 1978

[c] Normal range from Greene C E 1975

[d] Values determined by the Department of Haematology, South African Institute for Medical Research, Johannesburg

[e] Values determined by Clinical Laboratories, Jeppe Street, Johannesburg

mass and given over the following 24 hours. A bolus of furosemide** was infused intravenously at the rate of 1 to 2 mg/kg body mass. Antibiotic and Vitamin B Complex§ were administered to each patient at the same dosage rate as above.

The initial therapy outlined above was administered during the first 2 to 4 hours after presentation and varied subsequently as to the response of each individual patient. A favourable response was usually evident within 2 hours of the commencement of therapy and was assessed by the patient becoming more alert and less dyspnoeic.

Case 10 responded less favourably, and the blood transfusion was followed by the infusion of a low molecular weight dextran+ at the rate of 10 mℓ/kg body mass together with Plasmalyte B*.

A second bolus of intravenous heparin, at the above dosage was repeated after 4 to 6 hours in Cases 8, 9, 10, 11 and 12.

Cases 7, 8 and 10 also received 20 % manitol solution++ at the rate of 1 gm/kg body mass intravenously, during the post transfusion period.

Cases 8 and 10 in addition received heptaminol+++ at the rate of 5 mg/kg body mass intravenously.

Cases 10 and 12 also received 5 mℓ of essential phospholipids[a] diluted in a 5 % Dextrose solution*, post transfusion.

Subsequent therapy was determined by the response of each patient. Fluid therapy, using either Plasmalyte B* or 5 % Dextrose*, was usually continued for 3 days with the daily dosage varying between 40 and 80 mℓ/kg body mass. Antibiotic, Vitamin B Complex§ and prednisolone[b] were administered daily at the above dosage rates.

Icterus was detected in case 10 on the third day of hospitalization. This complication responded in 48 hours to supportive therapy which included 5 % Dextrose* and essential phospholipids[a].

Heparin[c] was infused twice daily at the above rate on the day following transfusion in Cases 8, 9, 10, 11 and 12 while it was continued for 3 days in Cases 8, 10 and 11 and 4 days in Cases 8 and 11.

An anabolic steroid[d] was administered intramuscularly on the second day of hospitalization, to case 12 at the dosage rate of 0,5 mℓ/4,5 kg body mass.

Cases 9, 10 and 12 ate meat 24 hours after the commencement of therapy while Case 11 began eating after 48 hours. Case 8 remained anorexic and was force fed. Because Cases 8 and 11 remained anorexic longer than 24 hours a gastrointestinal antacid[e] was administered orally at the approximate rate of 0,5 mℓ/kg body mass divided into 3 equal doses.

RESULTS

The response to therapy of the dogs in the mild category was excellent and all the dogs made an uneventful recovery. The response of the dogs in the severe category was less dramatic. Two of the dogs (Cases 7 and 8) died. Case 7 died 4 hours after presentation. Case 8 responded initially to therapy but died 5 days after presentation.

Wherever possible, and with due consideration to the cost involved, serial observations were made on subsequent blood specimens in order to monitor the influence of therapy on the disease process (Table 1).

The prothrombin time (PT) was abnormally prolonged only in Case 10 and this returned to normal within 24 hours of the commencement of therapy.

The activated partial thromboplastin time (APTT) was abnormally prolonged in Cases 1, 4, 7, 8, 10, 11 and 12. Where serial determinations were made, the APTT returned toward the normal value once therapy was commenced in Cases 1, 8 and 10 but remained prolonged in Case 11.

Fibrinogen (Fib) levels were elevated in Cases 1, 2, 4, 5, 7, 9, 11 and 12 at initial examination. Where serial determinations were made the level returned toward the normal level in Cases 2 and 11 but remained elevated in Case 8.

Fibrinogen degradation products (FDP) were elevated at initial examination only in dogs with severe disease (Cases 7, 8, 9, 10 and 11) and these levels returned toward the normal range once therapy was instituted.

A normal leukocyte count at initial examination was present in Cases 3, 6, 8, 9, 10, 11 and 12 which after treatment was commenced, remained within the normal range in Cases 6 and 11 but resulted in a leukocytosis (absolute neutrophilia) in Cases 8, 9, 10 and 12 with neutrophil counts of 19,8; 18,96; 23,166; 18,24 x $10^9/\ell$ respectively. Case 10 also developed an absolute monocytosis with a monocyte count of 1,8 x $10^9/\ell$. Although the leukocyte count remained within the normal range in case 11 an absolute neutrophilia (12 x $10^9/\ell$) and monocytosis (1,8 x $19^9/\ell$) developed after therapy began. Case 3 had an absolute monocytosis (1,65 x $10^9/\ell$).

A leukocytosis was present in Case 1 at initial examination as an absolute neutrophilia (16,732 x $10^9/\ell$).

A leukopenia was present at initial examination in Cases 2, 4, 5 and 7. A relative neutrophilia was present in Case 5. Following therapy a normal leukocyte count was found in Cases 2, 4 and 5.

A normal erythrocyte count (RCC), haemoglobin concentration (Hb) and packed cell volume (PCV) was present at initial examination in Cases 2, 5 and 7 but decreased slightly in cases 2 and 5 following therapy. Anaemia (decreased RCC, Hb, PCV) was present at initial examination in Cases 1, 3, 4, 6, 8, 9, 10 and 11 and improved following therapy in Cases 1, 6, 8, 9 and 10 but deteriorated in Cases 4 and 11. A raised PCV was present in Case 12 and this returned to the normal range once therapy was initiated.

The presence of intravascular haemolysis, assessed macroscopically as a pink discolouration of the plasma, was determined in all 12 dogs. All the dogs in the mild category had a negative test while 5 of the 6 dogs (Cases 7, 8, 10, 11 and 12) in the severe category were positive. Once therapy was initiated the test became negative within 24 hours.

Case 12 developed an oedema of the scrotum on the fifth day of hospitalization, and subsequently after discharge, 14 days after the initial therapy, a slough 2 centimeters in diameter was removed from the ventral scrotal skin. The ulcer healed uneventfully.

* Baxter
** Lasix - Hoechst
§ Centaur
+ Reomacrodex 40 - Saphar
++ Oxmitrol - Baxter
+++ Cortensor-Warner

[a] Hepavet 303 - Hoechst
[b] Deltacortril - Pfizer
[c] Pulvarin - Allen & Hanburys
[d] Vebenol - Ciba
[e] Amphogel - Wyeth

DISCUSSION

The therapeutic regime varied markedly between the two categories of infected dogs. The clinical symptoms, coagulation and haematological findings dictated that the severe category required intensive treatment to counteract medical shock and reverse the disastrous consequences of disseminated intravascular coagulation (DIC) initiated by the *B. canis* parasitaemia.

Case 12 presents an interesting phenomenon infrequently observed in practice where a slough of a distal extremity develops after initial treatment for biliary fever. A similar phenomenon, termed acrocyanosis, occurs in some human patients with malaria complicated by DIC and is due to infarction caused by fibrin thrombi[11]. It is postulated that the dry gangrene observed in case 12 was a result of local vascular occlusion due to DIC.

The diagnosis of DIC in dogs of the severe category was based upon the observation of prolonged PT and APTT, thrombocytopenia and increased FDP[43 44]. DIC is a secondary phenomenon which may be initiated by a wide variety of primary pathologic entities[1 26 30 34 42 50]. The pathophysiologic effects of DIC tend to perpetuate and accelerate the process[54]. Whenever possible, the first therapeutic consideration in the treatment of DIC is the control or elimination of the primary disease which has initiated the coagulopathy. This alone may suffice to bring about resolution of DIC[26]. Berenil* was used as the babecide of choice in the mild category due to its rapid babecidal action, ease of administration, small therapeutic dosage and relative absence of toxic properties at therapeutic dosage in animals not exhibiting medical shock[3 21 46]. Trypan blue† was chosen as the babecide in patients of the severe category as it does not stimulate the parasympathetic nervous system and thus aggrevate the medical shock which is frequently present in these critically ill patients, nor does it possess a potentialy toxic effect on the CNS as do the aromatic diamidines[4 5 18 21 31 32 36 46 47 49 57].

The second important therapeutic consideration in the management of DIC involves the elimination of all contributary thromboplastic factors such as shock, haemoconcentration, metabolic acidosis, hypoxia, dehydration and haemolysis which promote further deposition of fibrin[17 26 54]. Patients of the severe category have previously been described as "poor-prognosis cases"[40] and it has been shown that a severe metabolic acidosis with varying degrees of respiratory compensation exists in these dogs[7 40]. Fluid replacement therapy using polyionic alkalinizing agents and blood transfusion have been advocated to reverse the medical shock and metabolic acidosis[7 40]. 5 % Dextrose has been recommended in biliary fever patients in an attempt to partially provide their caloric requirements[7]. Hepatic degeneration is a frequent finding in dogs with biliary fever[16 38 39] and the readily available source of metabolizable carbohydrate would stimulate hepatic regeneration and enhances a positive nitrogen balance in an anorexic patient[19]. In the severe cases presented, shock, intravascular haemolysis, kidney failure and DIC were present[43 44] and these complications were treated with intravenous fluids which resulted in a prompt response in the majority of cases. In addition, Case 10 received a low molecular weight dextran to expand the plasma volume and further has the properties of decreasing the viscosity of blood, opening the microcirculation and decreasing platelet aggregation[17].

Heparin has been advocated in the treatment of DIC particularly during the initial hypercoagulable phase when it prevents coagulation[1 8 14 17 30 34 42 50 54]. The dosage recommended is 50 to 150 units/kg body mass given at intervals of 4 to 6 hours as an intermittent bolus or by continuous infusion, although higher doses may be required in the dog[17 30]. In general, heparin is indicated when the coagulopathy is itself life threatening before the primary disease can be completely corrected or when no effective treatment exists for the primary disease[26]. The majority of dogs in the present series of severe cases responded promptly to heparinization and it is reasonable to speculate that successful treatment of shock with fluid therapy would have been sufficient to control the DIC, without adjunctive heparin therapy. However, Case 7 received no heparin and died, and it is therefore also reasonable to speculate that without heparin therapy the DIC continued unabated. In order to determine if heparinization is complete the whole blood clotting time or APTT should be measured and maintained at 1,5 to 2 times normal[1 17]. Successful therapy results in the return of the coagulation factors, platelets and FDP to within normal limits[8 17]. In the present series of severe cases heparinization resulted in a return of the coagulation profiles and platelet numbers toward the normal limits over a variable period of time.

A fresh whole blood transfusion was given to cases 8, 9 and 10 to alleviate the anaemia, restore the platelet count and replace the coagulation factors. The danger of providing substrate and aiding coagulation and thrombus formation during the early hypercoagulable phase of DIC has been emphasized[1 8 14 17 42] and it is thus essential to heparinize the patient prior to replacement therapy[14 17]. Blood transfusion has been recommended as an adjuvant in the therapy of biliary fever[7 40] although the results have often been disappointing[40]. It is tempting to speculate that in the past, blood transfusion has given disappointing results because the patients were not heparinized and the transfusion potentiated the effects of DIC. It has been shown that antithrombin is the essential cofactor required for heparin activity and that some patients with DIC, refractory to heparin therapy, are often deficient in antithrombin[8 50]. Thus the replacement of depleted coagulation factors, in particular antithrombin, may be mandatory for successful treatment of DIC with heparin[50]. Blood transfusions are given to all biliary fever patients by the author, once the PCV falls to 10 % although in view of the above findings transfusion should be provided more readily. The risk of transfusion reactions at a repeat transfusion in the absence of freely available blood typing remains an obstacle, although plasma transfusions would provide the coagulation factors and reduce sensitization.

Antibiotic in the form of procain penicillin was administered routinely to avoid or minimize *Clostridium* spp hepatopathy which may arise subsequent to hepatic anoxia[19] as well as to provide a bacteriocidal antibiotic cover in a patient whose reticulo-endothelial system may be overburdened by the insults of the protozoan disease.

Glucocorticosteroid in the form of betamethasone was infused as an intravenous bolus to patients of the

* Hoechst
† Centaur

severe category for its vasodilatory effect to combat the tissue hypoperfusion of hypovolaemic shock[45]. Prednisolone was administered to all cases to minimize the immunologic erythrocyte destruction which is a significant factor in the development of the anaemia in biliary fever[38 56] as well as to stimulate erythropoiesis and megakaryopoiesis.

Vitamin B complex was administered as a supplement for the nutritional rehabilitation necessary for tissue repair in an acute febrile systemic disease in which a degree of hepatopathy frequently co-exists and in which the hepatic storage of vitamins could be deficient[19 38 39].

Diuretic in the form of furosemide was administered to decrease the pulmonary and cerebral oedema as well as to provide adequate urine flow and thus prevent acute renal failure. In selected cases the use of the osmotic diuretic, manitol, was used to reinforce the effects of furosemide particularly when severe cerebral oedema was suspected because of the aberrant nervous symptoms.

The positive inotropic agent, heptaminol, was used in selected patients when the medical shock was throught to be aggravated by a failing heart, auscultated as a rapid weak heart rate.

The use of essential phospholipids to provide nutrient for hepatic regeneration was used in selected patients and is advocated particularly in icteric patients who usually exhibit severe hepatic failure[39]. Anabolic steroid was administered to reduce protein catabolism, improve liver and kidney function and stimulate both erythropoiesis and appetite. A gastrointestinal antacid was administered to all anorexic patients to prevent vomition as a result of excessive gastric acid irritation.

CONCLUSIONS

Because *Babesia canis* infection complicated by DIC can be rapidly fatal, prompt diagnosis and an understanding of the dynamics of the process are essential for the intelligent management and treatment of the patient. For this reason it may be necessary to base therapeutic decisions on the results of a few rapidly available screening tests combined with the clinical findings in the patient. Heparin is not a panacea in the treatment of DIC and to be effective it must be used in conjunction with supportive therapy aimed at correcting the primary coagulation stimulus and other provocative forces which contribute to the demise of the homeostatic coagulation mechanisms.

ADDENDUM

Since the completion of this study it has been found that some dogs treated with a 1,5 % Trypan blue solution* at the dosage rate of 7,5 mg/kg, relapsed 3 to 42 days after initial treatment. The specific babecidal therapy for patients of the severe category has thus been amended. All patients exhibiting nervous symptoms and/or who exhibit profound medical shock are treated with a freshly prepared, sterile, 1 % Trypan blue solution given intravenously at the dosage rate of 1 mℓ/kg body mass (10 mg/kg). All other patients receive Berenil† subcutaneously at the dosage rate of 3,5 mg/kg body mass as a 7 % solution. Those patients receiving Trypan blue initially are treated with Berenil on Day 3 to 5 of hospitalization, once their condition has stabilised.

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† Hoechst

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SOME ASPECTS OF THE EPIDEMIOLOGY AND CONTROL OF BOVINE BABESIOSIS IN AUSTRALIA

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ABSTRACT: Callow L.L. **Some aspects of the epidemiology and control of bovine babesiosis in Australia.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 353-356 (En) Queensland Department of Primary Industries, Animal Research Institute, Tick Fever Research Centre, Wacol, Queensland 4076, Australia.

A short account of the epidemiology and control of babesiosis in Australia is presented. Epidemiological topics discussed include differences in the transmission of *Babesia bovis* and *B. bigemina* by the cattle tick, *Boophilus microplus* and the relative prevalence, disease incidence and pathogenicity of *B. bovis* and *B. bigemina*. Circumstances under which babesiosis occurs in Australia are described. In the Section on control, only vaccination is discussed. Changes in the preparation of babesial vaccines, particularly those resulting in a highly infective vaccine containing relatively avirulent *B. bovis* are described. Fluctuations in demand, such as the increase from about 100,000 to over 1,000,000 doses in 4 years in the mid-1960s are shown. An unexpected increase in the use of *A. centrale* in 1973 is discussed, and the supply of *B. bigemina* for cattle exported from Australia reported.

INTRODUCTION

In this review, aspects of the occurrence and control of babesiosis in Australia will be considered. In discussing the epidemiology I will describe the transmission and prevalence of *Babesia bovis* and *B. bigemina,* show a relationship between disease incidence and the pathogenicity of the organisms, and will summarize our understanding of the causation of disease. The Section on control will be mainly devoted to developments in the preparation and use of vaccine over the last 15 years.

EPIDEMIOLOGY

The parasites

Until recently, the Australian *Babesia* of cattle were called *B. argentina* and *B. bigemina.* For the last 2–3 years we have not used *'B. argentina',* and have referred to the smaller, more pathogenic organism by its correct name, *B. bovis.* A series of studies have confirmed the synonomy[8 23] of *B. bovis, B. argentina* and probably *B. berbera.* There is increasing evidence that substantial cross-protection exists amongst strains of the same species, widely separated geographically.

Transmission

The common cattle tick, *Boophilus microplus,* is the only vector for *Babesia* in Australia. Infection of the tick with both species occurs as it ingests infected blood during the late feeding stage of the adult female. After transovarial transmission, interesting differences occur in the transmission of the two parasites to cattle in the next tick generation. For *B. bovis*, infectivity terminates by the end of the larval stage[30], but does not *start* for *B. bigemina* until midway through the nymphal stage[3 22]. Thereafter, transmission of *B. bigemina* appears to be continuous through the remainder of the nymphal stage and then by both female and male adult ticks. Examination of tick salivary glands indicates that replete females, and males up to 35 days old may be infective[14 15]. Epidemiological consequences of these patterns are that prepatent periods for *B. bovis* (a minimum of 6–7 days after tick attachment) are about 1 week shorter than for *B. bigemina,* and that *B. bigemina* may be more readily spread than *B. bovis* by transfer of tick stages, particularly adult males. Despite its classification as a one-host tick *B. microplus* transfers amongst cattle held in close proximity. Another possible consequence of the persistence of *B. bigemina* infectivity throughout the adult stage is that the almost replete tick may reinfect itself from its own infective salivary secretion. This would explain observations of ticks retaining infection with certain species of *Babesia,* when fed on unnatural hosts.

Prevalence

The serology of babesiosis has been studied intensively in Australia[9 19 25 28]. There are now reliable indirect haemagglutination tests and indirect fluorescent antibody tests for *B. bovis,* but tests for *B. bigemina* lack sensitivity and specificity. Comparisons of the prevalence of *B. bovis* and *B. bigemina* are therefore difficult, but experimental evidence that *B. bigemina* is the more prevalent organism has mounted. In an early study[27] of two herds in the enzootic area, in which thick blood films from calves were examined monthly from birth, infections with *B. bigemina* developed before infections with *B. bovis*, suggesting a greater prevalence of the former organism. Five groups of 20–30 calves born each year for the last 5 years at an experimental site 300 km north of Brisbane[20] have been monitored for the development of natural infections with *B. bigemina* (by fortnightly thick blood film examination) and *B. bovis* (by fortnightly thick film and serological examination). In general, *B. bigemina* has been detected in blood films much earlier and more frequently than *B. bovis.* To take into account the fact that *B. bigemina* is more easily found in blood films than *B. bovis*, by virtue of higher peripheral parasitaemias, the time of seroconversion less 7 days for *B. bovis* was compared with the time of the first demonstration of *B. bigemina* in a blood film. In 4 of the 5 years the calves were confirmed as having contracted *B. bigemina* before *B. bovis*[21]. Very recently, as part of another experiment, 14 susceptible animals were exposed to natural infection on a farm near Brisbane. When observations were terminated 19 days later, six had become infected with *B. bigemina,* three with *B. bovis* and five were still not infected[4].

Distribution and incidence of disease

B. microplus infests less than one third of the continent of Australia, being confined to warmer and moister

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regions of the north and east of the country. Babesiosis is enzootic in all infested areas except the small tract of country where *B. microplus* occurs in northern New South Wales.

In Australia, *B. bovis* causes many more outbreaks of disease than *B. bigemina,* and has done so for many years[24 26 35]. From diagnostic records of my laboratory for the period 1973–1978 (Table 1), *B. bovis* consistently caused approximately nine times as many outbreaks as *B. bigemina.*

Table 1: PROPORTION OF BABESIOSIS CAUSED BY *BABESIA BOVIS* BY YEAR, 1973–1978

	1973	1974	1975	1976	1977	1978
Total outbreaks confirmed	128	131	88	138	91	59
% due to *B. bovis**	86,7	92,4	83,0	90,6	89,0	93,2

Average 1973–1978 = 89,15 %

Table 2: MORBIDITY AND MORTALITY DUE TO *BABESIA BOVIS* OR *BABESIA BIGEMINA* IN WELL-DOCUMENTED OUTBREAKS OF BABESIOSIS DURING 1974–1976

	Number of Outbreaks	Cattle at Risk	% Sick	% Dead
Babesia bovis	178	16 884	1,92	2,49
Babesia bigemina	21	2 222	1,98	0,72

Two effects probably contribute to the difference in disease incidences. One is that the greater prevalence of *B. bigemina* results in higher levels of enzootic stability[29 31] for this organism and fewer cattle being at risk. The other is that *B. bigemina* is less pathogenic than *B. bovis* in Australia. In a study of 199 well-documented outbreaks of babesiosis during 1974–1976, the mortality rate due to *B. bovis* was 2,49 % against 0,72 % due to *B. bigemina* (Table 2). Other observations have suggested that infections with *B. bigemina* are unlikely to be lethal, even in fully susceptible cattle introduced to the enzootic area. In an experiment performed to confirm that *B. bigemina* might safely be left out of babesiosis vaccine, three groups totalling 32 susceptible steers 18–24 months of age were subjected to natural infection in different tick-infested environments. These developed parasitaemias with *B. bigemina,* but were not clinically affected[5]. Since then, very large numbers of cattle have been brought into the enzootic area, vaccinated only against *B. bovis,* and have survived despite inevitable, natural challenge with *B. bigemina*[34].

Circumstances leading to disease outbreaks

Most disease occurs in cattle bred within the enzootic area. Low prevalence of *Babesia* in some environments reduces the chance of cattle receiving an immunizing infection as young animals when they are normally relatively resistant[29 31]. Disease may result in uninfected cattle as they grow older, usually following increased exposure to ticks, which can result from seasonal change, relocation of the cattle or change in husbandry. The tick-infested status of cattle leads some owners to assume the herd is completely immune to babesiosis and therefore neglect vaccination. Less important circumstances leading to disease are epizootic spread of infected ticks to susceptible populations of cattle and introduction of susceptible cattle to the enzootic area. Cattle exposed under these conditions are often vaccinated because owners are aware of the risk, and losses are not as serious as they might be. Outbreaks of babesiosis in *Bos indicus* cattle and their crosses appear to occur less frequently than in European breeds.

CONTROL BY VACCINATION

Brief history of vaccination in Australia

The vaccines

Vaccination against babesiosis in Australia has been a worthwhile practice since 1897, although the vaccines have been modified a number of times over the years. A full history of the procedures is beyond the scope of this paper, and comprehensive reviews are available[1 2 36]. Nevertheless, some of the changes should be mentioned here. One of these was the use of *B. bovis* in vaccine after 1939; before that time *B. bigemina* was the only organism intentionally used as vaccine. Major changes in 1964 involved exclusion of *B. bigemina* from routinely used vaccine and the preparation of highly infective *B. bovis* vaccine in splenectomized calves[6]. Rapid syringe-passage of *B. bovis* in these hosts reduced its virulence[7] and allowed the production of sufficient parasites to meet an increasing demand. Before 1964, cattle assumed to be carrying babesial infections, following artificial infection and recovery ('bleeders'), had been used as donors of vaccine.

Vaccination against anaplasmosis cannot be divorced from vaccination against babesiosis. *Anaplasma centrale,* introduced from South Africa in 1934, was used in relatively small amounts, usually mixed with a babesial vaccine, until 1973. Demand for *A. centrale* has since increased from less than 5 % to approximately 60 % of vaccines supplied. This was not expected and the reasons are not clearcut, but probably include a belief, in 1973, that the incidence of anaplasmosis was increasing. In addition, the vaccine was recommended more positively from that time as a result of a changed method of production. As had been the case with *Babesia,* the blood of carriers of *A. centrale* was not always infective; preparation in splenectomized calves removed doubts concerning the infectivity of this vaccine.

Vaccination

Recommendations for use of vaccine have changed. Before 1964, vaccines were unreliable because of variable infectivity[10] of the blood from 'bleeders', and, to counteract this deficiency some owners revaccinated cattle several times. The practice continued after the vaccines were improved, and, with the greatly increased use of vaccination (Fig. 1), a problem with neonatal haemolytic anaemia in calves emerged[17]. After it became obvious that immunity following a single infection with *B. bovis* was of long duration[32 33], and repeated vaccination was unnecessary[18], more rational vaccination procedures were used. Where possible, cattle are now vaccinated once or twice before they are 12 months of age. There have been no new reports of neonatal haemolytic anaemia in vaccinated herds for several years.

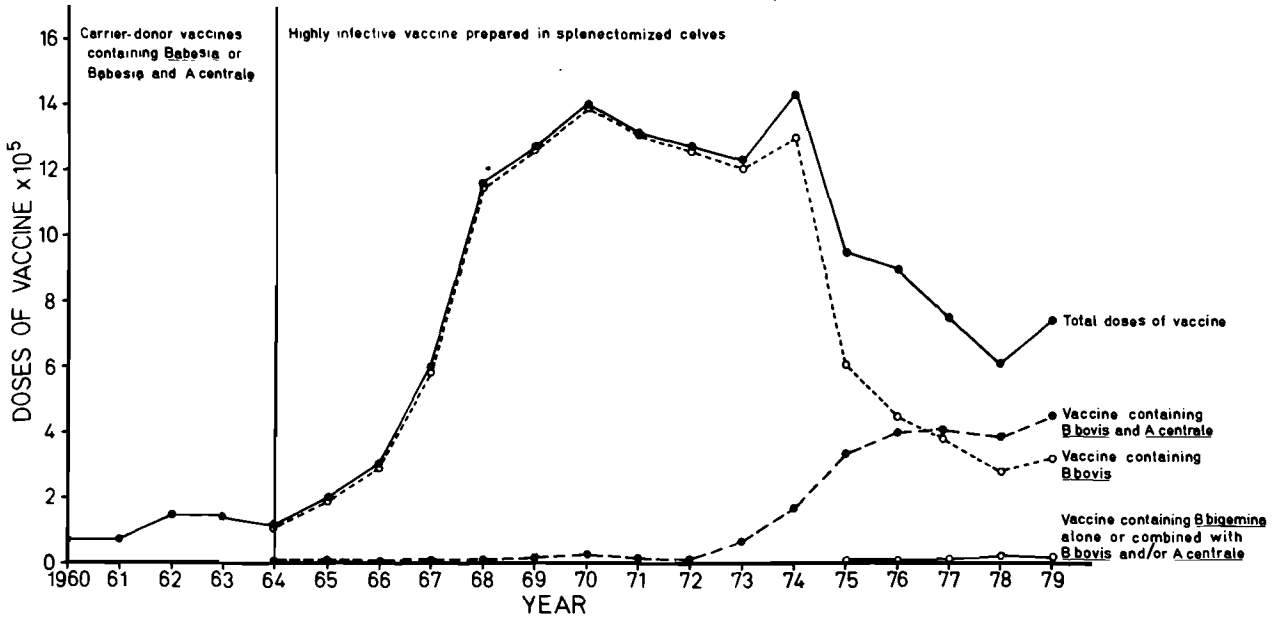

Fig. 1. Demand for vaccines against babesiosis and anaplasmosis in Australia, 1960–1979.

Demand for vaccine, 1960–1979

From Fig. 1, it is evident that the demand has fluctuated remarkably over the last 20 years. The 10-fold increase between 1964 and 1968 can be attributed to the widespread acceptance of the new vaccine. After 1974 when over 1,400,000 doses were supplied, demand fell away almost as quickly as it had risen during the mid-1960s. This coincided with the rapid collapse of the beef export market in Australia; despite the nominal cost of vaccine (A12 cents per dose at the time), owners did not value their stock sufficiently to continue vaccinating. A recent economic resurgence in the beef industry has seen an increase in demand for vaccine.

Other changes shown in Fig. 1 include the increased demand since 1973 for vaccine containing *A. centrale,* mentioned in the foregoing. During the last 6 years, in excess of 15,000 doses of vaccine containing *B. bigemina* have been supplied, mainly to vaccinate cattle being exported from Australia. This is in contrast to a demand of 1,420 doses for the 6 years preceding 1974, almost all of which was used in local herds experiencing disease due to *B. bigemina.*

Current research and development in vaccination

Because the method used since 1964 has allowed the production of effective, safe and relatively cheap vaccine, there are no plans to modify it significantly. We are now aware that the immunogenicity of vaccine strains of *B. bovis* may vary[2,16] and for the last 2,5 years have used only strains syringe passaged 20–30 times following their isolation; it appears that unlimited passaging may adversely affect the antigenicity of *B. bovis.* A method of reducing the virulence of *B. bigemina* used in vaccine has been developed[2,12]. Although no attempt is being made to develop an export market, we are sympathetic to some requests for vaccine from developing countries. To this end, vaccine has been cryopreserved ready for shipment, using techniques developed to store experimental and vaccine strains of *Babesia*[11,13].

ACKNOWLEDGMENTS

I wish to thank Len Mellors, Bill McGregor, Barry Rodwell and Gail Farlow for continuing efforts to improve and refine the vaccines during the 1960s and early 1970s. These people also bore the brunt of a rapidly escalating demand for vaccine, which had to be met before suitable facilities were available. Len Mellors prepared the figure. Our work has been supported by funds provided by the Australian Meat Research Committee and the Queensland Department of Primary Industries.

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EPIDEMIOLOGY AND CONTROL OF BOVINE BABESIOSIS IN SOUTH AFRICA

A.J. DE VOS

ABSTRACT: De Vos, A.J. **Epidemiology and control of bovine babesiosis in South Africa.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 357-362 (En) Section of Protozoology, Veterinary Research Institute, Onderstepoort, Republic of South Africa.

Babesiosis is the cause of serious economic losses in South Africa and involves most areas with an annual rainfall of more than 400 mm. Both *Babesia bigemina* and *Babesia bovis* are present and both are considered to be important. The vectors, distribution and factors influencing enzootic stability of both species are discussed. Except in some areas only marginally suitable for ticks, and where tick control alone may be adequate, control by immunisation is recommended. Short term prevention can be achieved by chemoprophylaxis.

INTRODUCTION

Babesiosis has been generally recognized as one of the most important cattle diseases in South Africa during the past decade or so[4] and, at present, 85 % of the total cattle population in this country is potentially at risk (De Vos, unpublished observations, 1978). It has been said to have caused the loss of an estimated 8 000 head annually in Natal alone as recently as 1971–72[2].

Two parasites are known to be involved in South Africa, viz. *Babesia bigemina* and *Babesia bovis*[12 15]. Although clinically difficult to differentiate, the diseases caused by these two parasites are so dissimilar in many other respects that they are generally considered to be separate entities and the names African and Asiatic redwater have come into common use. In this paper, a brief review will be given of our current knowledge and views on the epidemiology and control of these diseases.

EPIDEMIOLOGY

Transmission

Both *B. bovis* and *B. bigemina* are transmitted chiefly by *Boophilus* spp. in South Africa, but in very different ways. *Boophilus microplus* is the main, if not the only, vector of *B. bovis* (Thomas, 1972, personal communication; Potgieter, 1977[15]) while *B. bigemina* is readily transmitted by both *Boophilus decoloratus* and *B. microplus*[13 15 18]. Transovarian passage of both *Babesia* spp. occurs in the tick vector with transmission of *B. bovis* taking place in the larval stage[17 19] and that of *B. bigemina* in the nymphal and adult stages[15 18]. Potgieter & Els[18] also found that *B. bigemina* in these infected adults was capable of further uninterrupted development and even transovarial passage to further generations in the absence of reinfection.

In addition to the *Boophilus* spp. Thomas (unpublished observations, 1975) also found a strain of *Hyalomma rufipes* naturally infected with a parasite later identified as *B. bigemina*. When comparing the distributions of *B. bigemina* (Fig. 1) and *H. rufipes*[11], however, it seems highly unlikely that this tick species plays a signficant role in the epidemiology of African redwater.

Distribution

As can be expected, the distribution of both *B. bigemina* and *B. bovis* is intimately related to that of their main vectors. Of the 2 *Boophilus* spp present in South Africa (Fig. 1, 2), *B. decoloratus* is the most widespread and, as a result, *B. bigemina* (Fig. 1) has a wider distribution than *B. bovis* (Fig. 2). The most important factor limiting the distribution of this tick is decreasing humidity[9 22] and, in general, it is absent from areas with an average annual rainfall of less than about 380 mm (Fig. 1, 4). Although its numbers may be somewhat reduced by cold[9], *B. decoloratus* manages to maintain

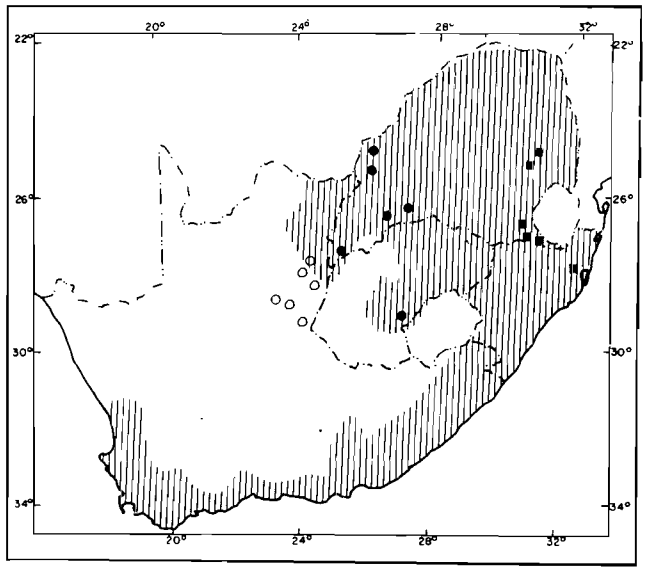

Fig. 1. Distribution of *Boophilus decoloratus* (vertical lines)[11] and *Babesia bigemina* in South Africa.

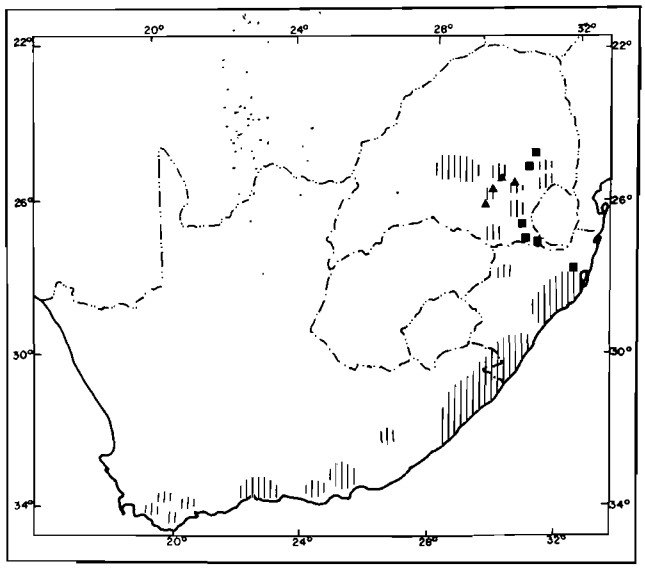

Fig. 2. Distribution of *Boophilus microplus* (vertical lines[11] and *Babesia bovis* in South Africa.

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itself in areas within the zone of 90 days frost spread over 150 days per annum (Fig. 1, 3).

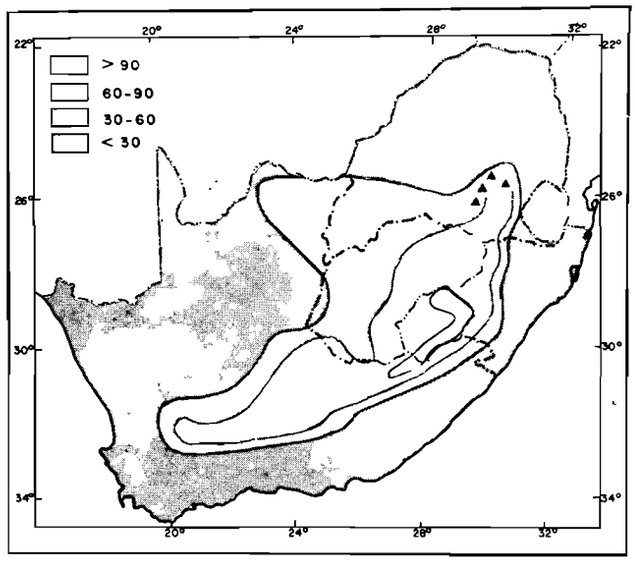

Fig. 3. Annual number of days of frost.

B. microplus, commonly regarded as exogenous[9], on the other hand, appears to be more susceptible to climatic extremes and in the field it – and by implication therefore also *B. bovis* – is most prevalent in the milder coastal areas (Fig. 2)[11]. It is, however, also present in highveld areas having up to 60 days of frost, spread over a period of up to 150 days per annum (Fig. 2, 3)[9]. This is in contrast to its known preference for warm, humid conditions in other parts of Africa but may be attributed to localized microclimatic conditions meeting the minimal conditions for survival. In general, it can be assumed that cold does influence its spread and survival[9]. In addition, it is limited by decreasing humidity; in Tanzania for instance, it is absent where the annual rainfall is below 500 mm[23].

Both *Babesia* spp are therefore widespread in the cattle producing parts of South Africa but are not of equal importance in all areas. A reason for this may be found in the so-called enzootic stability or instability of the diseases in various parts and their periodical appearance in others.

Enzootic stability and instability

Enzootic stability with regard to bovine babesiosis is defined by Callow, 1977[6] as the condition where there is frequent transmission of the parasites and infection of all animals occurs during the period that young animals are protected by passively acquired and non-specific factors, i.e. within the first 6 to 9 months of life. Acquired immunity develops without the host becoming obviously sick and local animals are therefore generally immune to the *Babesia* sp of spp involved and suffer minimally from this disease. A stable situation for one *Babesis* sp does not imply a similar situation for the other species (see below).

Enzootic instability on the other hand, defines the situation in which some animals in the herd fail to become infected for a considerable period after birth, i.e a host-parasite imbalance exists resulting from infrequent transmission[5,6]. Disease is then seen when susceptible animals in a herd encounter infected ticks.

The creation of unstable situations in South Africa is to a large extent dependent on 2 factors, viz. unfavourable climatic conditions and the injudicious control of ticks[4,10]. One or both of these factors may influence the stability of either of the *Babesia* spp in any given area.

Marked variations in the climate and geography within the enzootic areas of both *Babesia* spp in South Africa, as well as extremes in the efficacy of general tick control programmes, make generalizations of the status of the Babesias in various parts of the country virtually impossible. The following examples can, however, be given of the effect of tick control and climate on the enzootic status of both *Babesia* spp in a few selected areas:

1. In Natal as well as parts of the eastern Cape and the eastern Transvaal, regular control of ticks is essential to limit the numbers of problem species such as *Rhipicephalus appendiculatus.* In addition, regulations[1] were promulgated under the Animal Diseases and Parasites Act of 1956, according to which every owner of cattle in most of these parts must dip or spray his cattle every 7 days. Both *Boophilus* spp are well suited to survive in these areas. However, due to poor enforcement of these regulations and the development of acaricide resistance in some instances, as well as mediocre to very effective tick eradication in others, a very explosive situation has developed where some farms are clean, but at risk, some are stable enzootic at the other end of the scale, and many are unstable for one or both *Babesia* spp (Table 1).
2. Over most of the eastern Transvaal highveld more than 30 days of frost occurs annually (Fig. 3). Despite this, *B. decoloratus* appears to be firmly established in this region (Fig. 1) and, in the absence of adequate tick eradication programmes, a stable enzootic situation should therefore exist for *B. bigemina. B. microplus,* however, is severely limited by the cold winters and is not present throughout the region (Fig. 2). As a result, the situation for *B. bovis* is highly unstable (Table 2).
3. Due to unsuitable climatic conditions *B. microplus,* and therefore *B. bovis*, is absent from most of the Orange Free State and northern and western Transvaal. *B. decoloratus* on the other hand is usually well established in these parts and *B. bigemina* should therefore be enzootically stable unless the balance is upset by chemical tick control (Table 3) or drought conditions.

Cattle imported into an enzootic area

Severe losses often occur due to babesiosis when susceptible cattle are brought into an area where *Boophilus* spp. are prevalent. Mortality rates of 5–10 % have been recorded in such outbreaks (De Vos, unpublished observations, 1975).

Epizootic spread

As mentioned above, *B. decoloratus* requires about 380 mm of rainfall annually for survival. This renders a large part of South Africa from the southern Free State, the Karoo and northern Cape to the Atlantic ocean unsuitable for the survival of this tick (Fig 4).

Table 1: EFFECT OF TICK ERADICATION PROGRAMME ON THE ENZOOTIC STABILITY OF BOVINE *BABESIA* SPP IN THE HIGH RAINFALL AREA (FARMS MARKED ■)

District	*B. bigemina* % positive	*B. bigemina* Losses	*B. bigemina* Status	*B. bovis* % positive	*B. bovis* Losses	*B. bovis* Status	Tick control	Mean annual rainfall (mm)	No. of frosty days in winter
Piet Retief	30*	+	unstable	0*	–	at risk	good	884	<30
Piet Retief	40	+	unstable	27	++	unstable	good	884	<30
Wakkerstroom	100	–	stable	100	–	stable	poor	762	<30
Pelgrimsrust	5	?	unstable	10	+	unstable	good	950	<30
Lydenburg	100	–	stable	100	–	stable	poor	950	<30
Hluhluwe	0	–	at risk	0	–	at risk	good	?	<30

* Percentage of animals on farm serologically positive in 1–2 year age group

Table 2: EFFECT OF LOW TEMPERATURES ON ENZOOTIC STABILITY OF BOVINE *BABESIA* SPP (FARMS MARKED ▲)

District	*B. bigemina* % positive	*B. bigemina* Losses	*B. bigemina* Status	*B. bovis* % positive	*B. bovis* Losses	*B. bovis* Status	Altitude (m)	Number of frosty days in winter	Mean annual rainfall (mm)	Tick control
Witbank	83*	–	stable	53*	++	unstable	1 500	30–60	727	sporadic
Witbank	90	–	stable	0	–	at risk	1 500	30–60	727	handdressing
Witbank	73	–	stable ?	0	–	at risk	1 500	30–60	727	sporadic
Middelburg	85	–	stable	25	+	unstable	1 800	60–90	727	sporadic

* Percentage of animals on farm serologically positive in 1–2 year age group

Table 3: ENZOOTIC STABILITY AND INSTABILITY OF *BABESIA BIGEMINA* (FARMS MARKED ●)

District	*B. bigemina* % positive	*B. bigemina* Losses	*B. bigemina* Status	*B. bovis* % positive	*B. bovis* Status	Mean annual rainfall (mm)	Tick control
Marico	0*	–	at risk	0*	absent	567	good
Marico	63	?	unstable	0	absent	567	good
Potchefstroom	60	++	unstable	0	absent	591	good
Klerksdorp	43	+	unstable	0	absent	591	poor
Christiana	100	–	enzootic	0	absent	450	none
Wepener	98	–	enzootic	0	absent	612	poor

* Percentage of animals on farm serologically positive in 1–2 year age group

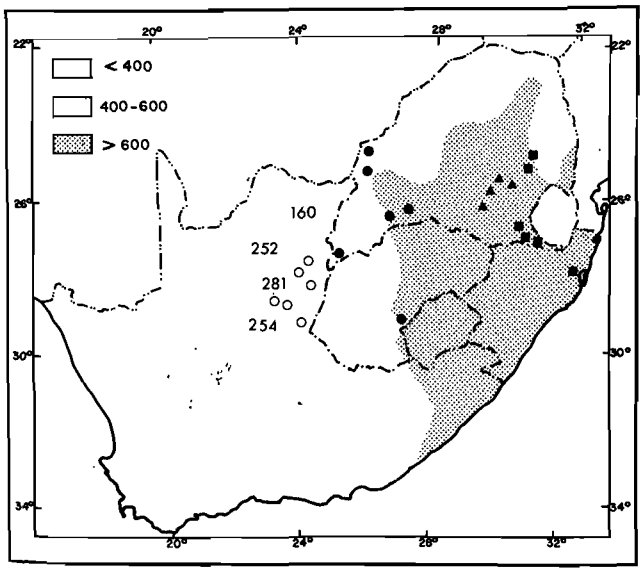

Fig. 4. Mean annual rainfall. The percentage increase above the normal for 1974 is given for 4 districts with an annual mean of less than 400 mm.

From 1974 through 1976 unusually high rainfall was recorded in this generally dry part of the country. The percentage increase above the normal for 1974 is illustrated in Fig. 4. This resulted in the creation – presumably temporarily – of a suitable environment for *B. decoloratus*, in this previously uninfested country, and consequently a westerly movement of the *B. decoloratus* – *B. bigemina* cut-off line. Although this led to large outbreaks of babesiosis, the presence of *B. decoloratus* on a farm appeared to be dependent on active introduction and it was not present throughout the region (Table 4). With rainfall figures returning to normal levels from 1977, it seems unlikely that babesiosis will remain a problem for long in this part of the country.

Annual incidence

Boophilus spp. are one-host ticks active mainly during the warmer months i.e. from September to June, in South Africa[11], but may be active throughout the year in warmer areas. As a result the vast majority of cases of babesiosis occur during the summer months.

Table 4: EPIZOOTIC SPREAD OF *BABESIA BIGEMINA* (FARMS MARKED ○)

District	*B. bigemina*		*B. bovis*	Mean annual rainfall (mm)
	% positive	Losses	% positive	
Barkly West	96*	+	0*	377
Barkly West	86	+	0	377
Barkly West	0	–	0	377
Herbert	0	–	0	282
Herbert	0	–	0	282
Herbert	0	–	0	282

* Percentage of animals on farm serologically positive in 1–2 year age group

Breed susceptibility

As outlined by Bigalke, 1976[3] substantial evidence exists that *Bos indicus* (e.g. Afrikaner) breeds carry smaller tick burdens and there is some evidence that they also have a higher level of resistance against some tick-borne diseases than *Bos taurus* breeds. Further evidence was supplied by De Vos (unpublished observations, 1978) when he compared 2 herds, 1 Afrikaner and the other Simmenthaler, born and bred on the same farm, kept under similar conditions and of the same age group (Table 5).

Table 5: RELATIVE INFECTION RATES OF *B. BIGEMINA* AND *B. BOVIS* IN 2 BREEDS OF CATTLE KEPT UNDER SIMILAR CONDITIONS ON THE SAME FARM IN THE WESTERN TRANSVAAL

	B. bigemina		*B. bovis*
	% positive	Losses	% positive
Bos indicus	33*	–	0*
Bos taurus	60	++	0

* Percentage of animals serologically positive in 1–2 year age group.

CONTROL

Eradication of ticks is the most desirable solution to the problem of tick-borne diseases in general, but Bigalke, 1976[3], recently considered it quite unrealistic for several reasons to attempt eradication with the aid of chemical acaricides on a national scale in South Africa. He considered that the only alternative for the foreseeable future would be to live with ticks and their diseases, i.e. to learn to live with nature, rather than against it. This was a long-term approach that could be achieved by the strategic use of acaricides, the application of vaccines in enzootically unstable situations and by farming with cattle naturaly resistant to ticks.

Tick eradication

During a survey of 31 farms in the enzootic areas for one or both *Babesia* spp. De Vos (unpublished observations, 1979) found control of *Boophilus* spp. to be good on 18 (58 %) of the farms. Based on serological evidence, however, as summarized in Tables 1 and 3, eradication of babesiosis was apparently achieved in only 2 instances while in 14 a potentially stable situaton was converted to one of instability with confirmed or suspected losses due to babesiosis.

Total eradication of babesiosis from a farm in the enzootic region is therefore by no means an easy task – and the risks involved in maintaining a susceptible herd in that part of the country are obvious. The control of babesiosis, and other tick-borne diseases by tick control alone, as prescribed by some instances, is therefore no solution to the problem[10] where *Boophilus* spp. are well established.

In some of the drier parts of the country, on the other hand, where *B. bigemina* occurs epizootically and the climate is not continually favourable for the survival of *B. decoloratus* (Table 4, Fig. 3), tick eradication may be a solution to the problem[10].

Where tick control is necessary to suppress problem species such as *R. appendiculatus,* or is enforced by law as outlined above, it must be remembered that this may well create a dangerous unstable situation for one or both *Babesia* spp. Under these conditions the only way to stabilise the situation is to make use of a vaccine.

Vaccination

The protection of cattle against *B. bigemina* by immunization was used in South Africa as early as 1912. It was done by inoculating animals with blood known to be infected with both this parasite and *Anaplasma centrale*[21]. This vaccine was produced in Pretoria and Grahamstown and issued as 5 mℓ doses costing one shilling per dose.

Composition of the vaccine today:

The vaccine is at present produced only at the Veterinary Research Institute, Onderstepoort, and consists of pooled blood collected from animals acutely infected either with *B. bovis* or *B. bigemina.* Both infections are quantitated and adjusted to ensure that each 2 mℓ dose of vaccine contains approximately 1 x 10^7 parasites of each species at the time of despatch[7]. The blood is collected in anticoagulant citrate dextrose (ACD) solution and penicillin (200 u/mℓ) and streptomycin (100 μg/mℓ) are added as preservatives.

Directions for use:

Blood is collected on demand and issued weekly. Orders should consequently be placed well in advance. Since the organisms deteriorate rapidly, the vaccine must be used within 6 days of issue, i.e. not later than the expiry date printed on the label. On receipt the vaccine should be kept in a refrigerator and injected as soon as possible. The vaccine must not be frozen. It is available in quantities of 2, 5 and 25 doses. The dose of 2 mℓ is injected subcutaneously irrespective of the size of the animal.

There is some attenuation of the strains used in the vaccine but it is by no means avirulent. A general recommendation is therefore to limit the use of the vaccine to calves when non-specific resistance will minimize the risk of vaccine reactions.

When older animals have to be vaccinated, i.e. when susceptible animals are introduced into a known or suspected enzootic area, the necessary precautions will have to be taken. Ideally these will consist of the observation of vaccinated animals and taking of temperatures daily for 21 days after vaccination. Reacting animals should be treated with a suitable babesiacide. As both *Babesia* spp. are present in the vaccine, 2 reactions may be seen. Most of the animals will react to *B.*

bigemina first while about 40 % may later react to *B. bovis* as well.

Where this procedure is deemed impractical, as is often the case when large numbers of animals are to be vaccinated, blocking therapy can be used. Diminazene used 7 days after vaccination is particularly effective in this respect. This will ensure that a durable immunity develops against *B. bovis* but revaccination 2–3 weeks later is advised to establish immunity to *B. bigemina* if this is required. The latter procedure also applies to cases where control of vaccine reactions was necessary and babesiacides other than euflavine were used. If possible, pregnant cows should not be vaccinated.

Protective immunity develops in 3–4 weeks[4]. In the absence of adequate natural challenges, antibody titres in a vaccinated herd will drop in time (De Vos, unpublished observation, 1979) and progressively more animals will become serologically negative (Table 6). Despite this, protective immunity after a single vaccination in the case of *B. bovis* appeared to last for several years (De Vos, unpublished observations, 1977). The duration of immunity to *B. bigemina* is unknown but appears to break down in time in the absence of natural challenge (Neitz, 1969). Despite this, revaccination of animals is not a procedure often advocated in South Africa, even under conditions of minimal tick challenge.

Table 6: PERCENTAGE OF ANIMALS SEROLOGICALLY POSITIVE AT VARIOUS INTERVALS AFTER VACCINATION

	B. bigemina	*B. bovis*
Unvaccinated >36 months old	30*	10*
2 months post vaccination	93	97
9 months post vaccination	100	93
14 months post vaccination	73	73
21 months post vaccination	60	60
34 months post vaccination	57	43

* Survey conducted on Northern Farm, City Council of Johannesburg 1979 using the IFA technique. All animals were vaccinated at 4–5 months of age and positive reactions at serum dilutions of 1:40 were taken as positive.

Control of contamination:

To minimize the inadvertent spread of haemotropic diseases with this blood vaccine, only splenectomized 12–18 month old cattle born and raised at this Institute under strict tick-free conditions are used for the production of vaccine. Measures taken to control the spread of viral and bacterial diseases as well as leucosis are similar to those taken for the Onderstepoort anaplasmosis vaccine[16].

Haemolytic disease or neonatal isoerythrolysis:

This is a condition of the new-born calf caused by the action of isoimmune blood group antibodies of maternal origin[8]. The use of the current South African babesiosis vaccine consisting of approximately 0,5 mℓ of packed red blood cells has been shown to cause the development of antibodies to the relevant blood groups in inoculated cattle[14]. Despite this, the presence of haemolytic disease has not been confirmed in South Africa. Reasons for this may be the general practice of vaccinating cattle once only as calves before weaning age, thereby maximizing the interval between the dates of vaccination and calving, and the use of about 75 % of all babesiosis vaccine issued concomitantly with the anaplasmosis vaccine[14]. Despite this, veterinarians should be aware of the possibility of haemolytic disease occurring, albeit in a small percentage of calves. It must be stressed, however, that the advantages of the babesiosis vaccine to the cattle industry of this country are such that they minimize the importance of possible haemolytic disease as a factor worthy of consideration when the use of the vaccine is contemplated[14].

Chemoprophylaxis

This method of short-term control is often used in South Africa, particularly in the case of large outbreaks with high morbidity rates. Treatment of all affected and exposed animals with a suitable compound is a rapid and practical way to prevent further losses. Two drugs with prophylactic properties are available in South Africa. Imidocarb will protect cattle against *B. bovis* for about 4 weeks and against *B. bigemina* for at least 2 months[20]. Diminazene is effective against *B. bovis* for 2 weeks and against *B. bigemina* for about 4 weeks (De Vos, unpublished observations, 1977).

Short-term chemoprophylatic control is also useful in instances such as the transportation of susceptible animals through an affected area, temporary residence of such animals in an affected area and where pregnant cows are at risk.

The use of prophylactic compounds may be combined with an intensive dipping programme aimed at the eradication of the vector ticks, or subsequent vaccination to stabilize the situation. After prophylactic therapy with imidocarb, vaccination should be delayed for 4 weeks if immunity to *B. bovis* is required. If immunity to *B. bigemina* is also necessary, a further vaccination 2 months after therapy is indicated. When diminazene is used, immediate vaccination will ensure immunity to *B. bovis*. For immunity for *B. bigemina,* vaccination should be delayed for 3–4 weeks.

Tick resistant cattle

As outlined above *B. indicus* cattle carry smaller tick burdens than European breeds and also appear to be more resistant to babesiosis. South Africa is in the fortunate position with regard to *B. indicus* cattle in having the Afrikaner as the most popular beef breed (Osterhoff, 1974 cited by Bigalke, 1976[3]). In addition, 3 very productive beef breeds with a satisfactory *B. indicus* content are available in the Drakensberger, Bonsmara and Santa Gertrudis[3]. According to Bigalke, 1976[3], a much wider use of the latter 3, or similar breeds, in tick-infested regions are indicated and the development of a "milking Zebu" should receive the highest priority.

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EPIDEMIOLOGY AND CONTROL OF ANAPLASMOSIS IN AUSTRALIA

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ABSTRACT: Rogers R.J.; Shiels I.A. **Epidemiology and control of anaplasmosis in Australia.** *Journal of the South African Veterinary Association* (1979) **50** No 4 363-366 (En) Queensland Dept. of Primary Industries, Animal Research Institute, Yeerongpilly, Queensland 4105, Australia.

Anaplasmosis occurs in those areas of northern and eastern Australia infested by the cattle tick *Boophilus microplus* but it has been studied intensively only in Queensland. Anaplasmosis is predominantly a disease of autumn and winter and of cattle greater than 1 year of age. The complement fixation test has been used in serological surveys of the tick-infested areas of the state. Both clinical and subclinical infections occur only in tick-infested areas and they are both more frequent in *Bos taurus* than in *Bos indicus* cattle probably due to the greater susceptibility of the former to ticks. Prevalence of infection is significantly greater in cattle exposed to heavy tick infestations than it is in cattle exposed to light tick infestations. *B. microplus* is considered to be the main vector with transmission being effected by transtadial and intrastadial but not transovarial means. Transtadial transmission by *Rhipicephalus sanguineus* has been demonstrated but attempts to demonstrate transmission by *Haemaphysalis longicornis* were unsuccessful.

Vaccination with *Anaplasma centrale* is employed either as a routine preventative measure in young cattle or in the face of an outbreak. Attempts to attenuate a strain of *A. marginale* by adapting it to sheep were unsuccessful. Oxytetracycline and imidocarb have been used successfully to control the clinical disease.

INTRODUCTION

Anaplasmosis occurs in those areas of northern and eastern Australia infested by the cattle tick *Boophilus microplus* but it has only been studied in any detail in Queensland. *Anaplasma marginale* was first reported in Australia in 1933 by Legg [6] who had little difficulty in isolating strains of the organism by splenectomising naturally tick-infested calves in North Queensland. Legg further suggested that *A. marginale* was probably present as early as 1911 and may even have been introduced with 12 tick-infested Brahman cattle shipped to Darwin from Java in 1872.

THE NATURAL DISEASE

Data associated with field outbreaks have been recorded in southern Queensland over the period 1967 to 1978. Similar records for northern Queensland are incomplete because of a laboratory fire.

The seasonal distribution of outbreaks of Anaplasmosis differs from that of Babesiosis in that the majority outbreaks occur during autumn and winter. Fourteen percent of 402 outbreaks occurred in summer, 42 % in autumn, 36 % in winter and 8 % in spring. Maximum populations of *B. microplus* develop during autumn and the extension of outbreaks over winter is probably associated with the relatively long pre-patent period following tick-induced infections. This period varied from 27 to 47 days following inter and intrastadial *B. microplus* induced infections[2].

Analysis of 277 outbreaks where the age of cattle affected was known revealed that only 6,9 % were less than 1 year old, 37,9 % were 1 to 3 years old, and 55,2 % were greater than 3 years of age. This is not surprising since there is a well established reverse age immunity in anaplasmosis[4]. It does, however suggest that considerable numbers of cattle are not exposed to *A. marginale* until relatively late in life. The fact that 79,5 % of 259 outbreaks affected female cattle initially suggests greater susceptibility of that sex. However, a more likely explanation is that this bias is due to a combination of a greater proportion of females in the population[12] together with a marked age susceptibility. There is no obvious bias attributable to sex in *Babesia bovis* outbreaks (unpublished data) but only 17 % of Babesiosis outbreaks affect cattle greater than 3 years of age[10].

The breeds of cattle in 273 outbreaks of anaplasmosis were known and examination showed that dairy breeds were involved in 134 cases and beef breeds in 139 even though the ratio of dairy to beef breeds in the area is 1:7. Closer observation of dairy cattle resulting in detection of more clinical cases may be largely responsible for this. A detailed breakdown of the actual breeds of cattle involved is shown in Table 1. The dairy breed most frequently involved is the Australian Illawarra Shorthorn (AIS) and Herefords are the most frequently affected beef breed. While the distribution of outbreaks amongst dairy breeds reflects the relative proportions of individual breeds within the population the same relationship does not hold true for beef cattle. *Bos indicus* type crossbred cattle (Brahman X) comprises over half the beef cattle population but are only involved in 7,9 % of all beef cattle outbreaks. In contrast Herefords were involved in 61,9 %. They are the predominant *Bos taurus* beef breed and undoubtedly the greater susceptibility of *Bos taurus* breeds in general and Herefords in particular, to *B. microplus*[14] is largely responsible for their majority involvement in field outbreaks.

If the *B. microplus* infested area of Queensland is divided into three zones as shown in Figure 1 so that the north zone is that area to the north of the 22nd parallel, the central zone that area between the 22nd and 25th parallels and the south zone that area to the south of

Table 1: BREEDS OF CATTLE INVOLVED IN 273 CLINICAL OUTBREAKS OF ANAPLASMOSIS IN SOUTHERN QUEENSLAND FROM 1967 TO 1978 INCLUSIVE

Dairy Cattle			Beef Cattle		
Breed	Number	%	Breed	Number	%
AIS	50	37,3	Hereford	86	61,9
Jersey	37	27,6	Shorthorn	11	7,9
Friesian	25	18,7	Brahman X	11	7,9
Guernsey	13	9,7	Murray Grey	4	2,9
Unknown	9	6,7	Devon	3	2,2
TOTAL	134		Other	6	4,3
			Unknown	18	12,9
			TOTAL	139	

0038-2809/79/04/0363-0366

the 25th parallel, some marked differences in incidence of clinical disease are apparent. Most clinical cases over the period 1967 to 1978 were seen in the south zone where there were 0,154 cases/10^3 animals. There were 0,047 cases/10^3 animals in the central zone and only 0,027 cases/10^3 animals in the north zone. This progessive decrease in incidence from south to north is partly due to the more extensive grazing practices and decreased surveillance in northern areas, however, it is also associated with different levels of infection in these zones. No clinical cases have been recorded from the tick-free areas of the State.

ASPECTS OF THE EPIDEMIOLOGY OF ANAPLASMOSIS

Serological surveys[12 15] have detected an insigificant level of reactors in tick-free areas of the State. Each of the three tick-infested zones shown in Figure 1 have been surveyed for the presence of reactors to the complement fixation test for anaplasmosis and the results are given in Table 2. A haphazard sampling procedure in which those sera which came to hand was employed in the north zone on two occasions[11 15]. The mean prevalence established by this procedure is not very accurate and the divergance in levels shown illustrates this point. Sampling in the central and southern zones was based upon the distribution of the cattle population utilising a procedure designed to give an accurate estimate of prevalence. Despite the difference in sampling procedure the surveys show a trend to rising prevalence from south to north. This is associated with a decline in clinical outbreaks probably as a result of increasing exposure of young naturally resistant animals[4].

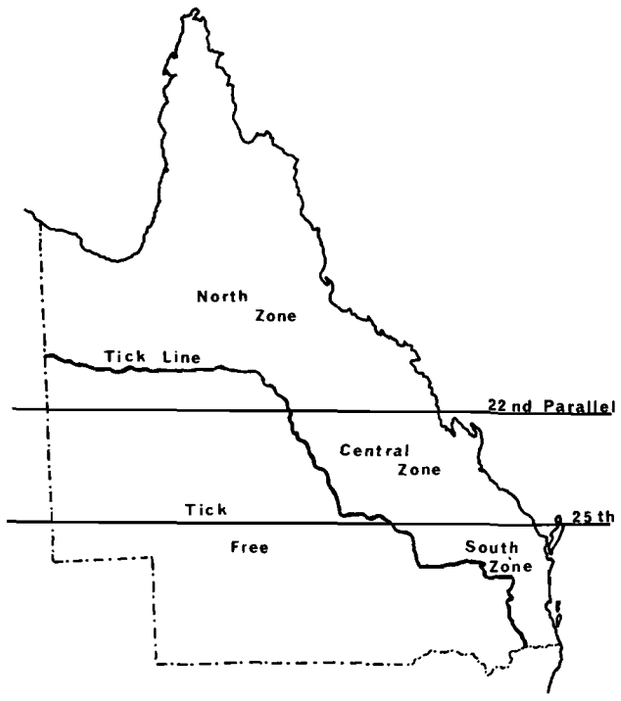

Fig. 1. Areas of Queensland surveyed for complement-fixing antibodies to *Anaplasma marginale*. Those areas to the south and west of the "tick line" are free from *Boophilus microplus*. The tick-infested area to the north and east of the "tick line" is divided into the north zone (above the 22nd parallel), the central zone (between the 22nd and 25th parallels) and the south zone (below the 25th parallel).

Table 2: PREVALENCE OF COMPLEMENT-FIXING ANTIBODIES IN CATTLE IN *BOOPHILUS MICROPLUS* INFESTED AREAS OF QUEENSLAND

Zone	% Reactors	No. of Sera
North (above 22nd parallel)	47,2	1 939
	63,1	531
Central (22nd to 25th parallel)	52,3	1 638*
South (below 25th parallel)	30,2	1 388*

* Sampling based upon distribution of the cattle population 0,4 % of 784 sera from cattle in tick-free areas reacted.

Statistical analysis of survey data[12] revealed that reactor prevalence was significantly greater in *Bos taurus* than *Bos indicus* cattle, that prevalence increased significantly with age, that there was no difference in prevalence attributable to sex, and that prevalence increased significantly both with stocking density and level of *B. microplus* infestation. Some of these findings are vector related in that *B. taurus* cattle have a higher prevalence and are more susceptible to *B. microplus*, higher stocking densities provide greater opportunity for transfer of partially developed ticks[13], and that heavier tick infestations are associated with higher prevalence levels.

TRANSMISSION

Clinical outbreaks of anaplasmosis have never occurred outside *B. microplus* infested areas in Queensland. This fact together with the observations described above provide strong evidence that *B. microplus* is the main vector of anaplasmosis in Queensland despite the failure to demonstrate transovarial transmission. It seems likely that transmission is effected mainly by transfer of adult males[2 5] and partially developed females[13] from host to host.

The possible vectors of anaplasmosis, which have been studied in Queensland, are shown in Table 3. March flies and stable flies have not been studied intensively but their failure to effect transmission when infected and susceptible splenectomised calves are penned together (unpublished observations) would support the contention of MacKerras et al[7] that they are not vectors. *Haemaphysalis longicornis* is found not infrequently on cattle but would appear to be incapable of transmitting *A. marginale*[3] whereas transtadial transmission by *Rhipicephalus sanguineus* a tick rarely, if ever, found on cattle, has been demonstrated[8]. However, it is unlikely that *R. sanguineus* plays a significant role in the transmission of anaplasmosis in Queensland.

CONTROL

Chemotherapy

Oxytetracycline at a dose rate of 10 mg/kg has been used extensively in Queensland and found to be highly effective in the control of Anaplasmosis. Two doses should be administered preferably intravenously, 24 hours apart and given early in the course of the disease. More recently long acting preparations of oxytetracycline have been found effective when administered as a single dose at 20 mg/kg[16]. Imidocarb, at dose rates vary-

Table 3: TRANSMISSION OF *ANAPLASMA MARGINALE* IN QUEENSLAND

Possible Vector	Intrastadial mechanical	Transtadial	Transovarial	Reference
Tabanus circumdatus *Stomoxys calcitrans*	–			MacKerras et al[7]
Boophilus microplus	+	+	–	Connell[1,2]
	(larvae, nymph; adult male and female)	+	–	Connell[1,2] Leatch[5]
Haemaphysalis longicornis	–	–	–	Connell[3]
Rhipicephalus sanguineus		+		Parker & Wilson[8]

Table 4: RESPONSE OF ANIMALS PREVIOUSLY VACCINATED WITH *BABESIA BOVIS, BABESIA BIGEMINA* AND *ANAPLASMA CENTRALE* TO CHALLENGE WITH *ANAPLASMA MARGINALE*

Group	n	Duration of* Parasitaemia (Days)	Maximum* Parasitaemia (%)	% fall** in PCV	Maximum* Temperature °C
Vaccinates	19	8,6 ± 6,1	4,7 ± 4,4	30,2 ± 19,6	39,6 ± 0,5
Controls	4	10,8 ± 6,3	10,4 ± 8,4	40,1 ± 23,4	40,7 ± 1,3

* Mean and standard deviation
** % fall from pre-inoculation level

ing from 2 to 5 mg/kg, administered by intramuscular injection has also been used to control anaplasmosis. It has the considerable advantage of being highly effective against *Babesia* thus avoiding the necessity of a possibly difficult differential diagnosis though it may be slightly less efficient against *Anaplasma* than oxytetracycline.

Vaccination

Protection has been conferred on susceptible cattle by infecting them with the less virulent *A. centrale* which was introduced from South Africa in 1934. Vaccination is performed either as a routine preventative measure in young cattle or in the face of an outbreak.The vaccine is prepared by passage in splenectomised calves, produces consistently mild reactions in the recipients and tests for transtadial and transovarial transmision with *B. microplus* have been negative (unpublished data).

There have been few suggestions that the vaccine has not withstood field challenge from *A. marginale,* however, a number of experimental observations have given cause for concern. An example of one such challenge experiment is shown in Table 4. Even though the control group was small it is apparent that the vaccinated animals reacted quite severely.

Because of this an attempt was made to produce an attenuated strain of *A. marginale* similar to that described by Ristic et al[9]. Parasitised blood was exposed to either 20 or 40 Krads of gamma irradiation in a Cobalt[60] source and then blind passaged, using 100 mℓ blood, at approximately 30 day intervals through two lines of spenectomised Merino sheep. Patent parasitaemias in the sheep were only detected during the ninth passage but transmission tests into splenectomised calves at passages 2, 5 and 10 were also positive. An agent giving protection to calves against challenge with virulent *A. marginale* was present at passage 20 but patency in the sheep had still not developed. Immunosuppressants including cortisone, antilymphocyte serum, cyclophosphamide and azathioprine were then used in recipient sheep without success and a transmission test to a splenectomised calf was negative at passage 26.

This failure to adapt an Australian strain of *A. marginale* to Merino sheep suggests that other means of attenuating *A. marginale* for vaccine purposes will have to be sought. Use of different breeds of sheep, prolonged rapid passage through splenectomised calves, or application of environmental stresses to infected vector stages are alternative approaches which might be considered.

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EPIZOOTIOLOGY AND CONTROL OF ANAPLASMOSIS IN SOUTH AFRICA

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ABSTRACT: Potgieter F.T. **Epizootiology and control of anaplasmosis in South Africa.** *Journal of the South African Veterinary Association* (1979) **50** No. 4 367-372 (En). Protozoology Section, Veterinary Research Institute Onderstepoort 0110, Republic of South Africa.

The history of bovine anaplasmosis, or tick-borne gallsickness, since the discovery of *Anaplasma marginale* by Sir Arnold Theiler is briefly reviewed. The development of the *Anaplasma centrale* vaccine by Theiler, up to the composition of the present vaccine issued by Onderstepoort in which the original isolate is still passaged, is discussed in detail.

Recent transmission studies at Onderstepoort have shown that 5 tick species are capable of transmitting anaplasmosis intrastadially, and intrastadial transmission, especially by adult male ticks, it is believed, could play an important role in the epizootiology of bovine anaplasmosis in South Africa.

Disease incidence and tick distribution are discussed in relation to enzootic and epizootic conditions.

Investigations have proved that the Onderstepoort *A. centrale* vaccine is not as avirulent in adult cattle as it was previously thought to be. The results of a field trial undertaken to test the infectivity of the vaccine, are given.

HISTORY OF ANAPLASMOSIS IN SOUTH AFRICA

Anaplasma marginale

Theiler[9] proved that the marginal points in the red blood cells of cattle was the causative organism of a specific disease belonging to the group popularly known in South Africa as gallsickness. He called the organism *Anaplasma marginale*[9 10 11]. Theiler[10], had seen these "marginal points" for a number of years (1905–1908) but stated that he was unable to determine their exact nature. Theiler[11] stated that, since the original literature[8], was not available to him at the beginning of his investigations, he described the organisms as "marginal points". He believed that *Babesia bigemina* and *A. marginale* had been confused because the organisms are transmitted simultaneously by the same tick *Boophilus decoloratus*[10].

Anaplasma marginale (var. *centrale*)

Attempts to isolate pure infections of *A. marginale,* indicated that animals from Aliwal North, although susceptible to redwater, seemed to react to blood containing "marginal points"[11]. It was concluded, therefore, that they would be best suited to isolate these "marginal points", which he subsequently did, by inoculating blood from these animals into imported cattle. A 3rd attempt with blood from Heifer 906 showed that the animals reacted very mildly to the infection and all recovered[14].

It is noteworthy that in "one or two cases", to use Theiler's own words, "the changes of the blood indicate a more severe anaemia due to heavy destruction of corpuscles, not sufficient, however, to cause death". This is an important observation and will be discussed elsewhere.

The causative organisms of these mild infections were found to be smaller than the ones described as *A. marginale.* This organism which was more centrally placed in the erythrocyte was designated *Anaplasma marginale* (variety *centrale*)[14].

Cross-immunity between *A. centrale* and *A. marginale*

Following this discovery, Theiler[14] immediately saw the potential in the use of such a non-virulent organism in a vaccine against *A. marginale* infections. In his first attempt 6 animals, which were immune to *A. centrale,* were challenged with *A. marginale.* All had mild reactions accompanied by the appearance of *A. marginale* in the blood smears. It was therefore concluded that the previous inoculation of *A. centrale* gave sufficient immunity to protect animals from severe attacks of anaplasmosis.

The problem of protecting imported cattle against redwater and anaplasmosis had to be tackled. Having demonstrated the protective cross-immunity to subsequent infection with the more virulent *A. marginale* in 39 heifers, it was found that redwater *(Babesia bigemina)* inoculation could be done before or after the anaplasmosis inoculation, however, it was more practical to do both at the same time[14]. The latter mode of vaccination is still being practised in South Africa.

The author of this paper would like to stress the fact that Theiler[14] never claimed that a complete immunity developed in vaccinated cattle but only that enough protection was provided to prevent death from, or evert the effects of, *A. marginale* infections.

Tick transmission of anaplasmosis

In a first experiment to prove tick-transmission of approximately 100 blue tick *(Boophilus decoloratus)* larvae from females collected of animals which were regarded as being immune to redwater and gallsickness, transmitted *A. marginale* so a susceptible heifer of a British breed[9]. A prepatent period of 75 days was recorded.

In his first report as Director of Veterinary Research, Theiler[14] gave a detailed report on the separation of anaplasmosis from babesiosis and the immunity conferred by *A. marginale* (variety *centrale*) against *A. marginale.* In this extension of this tick work, he found that *B. decoloratus* and *Rhipicephalus simus* were responsible for the transovarial transmission of anaplasmosis and that *B. decoloratus* transmitted both *A. centrale* and *A. marginale.* He reported only 1 experiment in which *R. simus* transmitted a pure infection of anaplasmosis transovarially but did not indicate whether it was *A. marginale* of *A. centrale.* Apparently Theiler[9 14] had no problem in transmitting anaplasmosis with these ticks. This aspect, and the fact that the incubation periods of the infections ranged from 55–114 days, have always been adversely criticized by those workers all over the world who have tried to duplicate his findings by using various strains of the parasite and different tick species. In South Africa there is no known record of any subsequent successful attempts at transmitting

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anaplasmosis transovarially with any tick species. It is still generally accepted that arthropod transmission of these parasites remains the least understood basic aspect of the disease.

A. centrale vaccine

Having obtained all these results, the first redwater and gallsickness vaccine was offered for sale[15]. Its dose consisted of a single injection of 5 mℓ to be given subcutaneously, and was sold for a shilling. It was indicated that the gallsickness reaction would not require any attention as it usually passed unnoticed.

Walker[16], who produced a genealogical chart, (p. 526) showing the mutability of *A. centrale*, found that the inoculation of susceptible cattle with redwater – gallsickness blood obtained from carrier animals did not always transmit a pure infection but did produce pure *A. marginale* infections in some cases. He concluded that a "mutation" from *A. centrale* to *A. marginale* might occur in passage through a susceptible animal.

Although this very interesting observation has never actually been pursued by any other worker in South Africa, the *A. centrale* isolate of Theiler has been passaged through vaccine donor cattle ever since it was isolated. No other record exists to our knowledge that the Onderstepoort *A. centrale* vaccine strain has ever reverted to a "dominant" *A. marginale* form.

Gallsickness

One interesting feature in the history of anaplasmosis is the use of the term "gallsickness". Theiler[12] pointed out that the term gallsickness used by the farmers is a collective name for a number of diseases of cattle that are characterized by symptoms indicating disturbances of the digestive organs. Post-mortem examinations usually show liver lesions and abnormal bile. He concluded that *A. marginale* is the causative organism of anaplasmosis or what he referred to as the original typical gallsickness. Robinson[7], who published a paper on non-specific gallsickness of cattle in South Africa, made the distinction between specific gallsickness, which he recognized as anaplasmosis, and non-specific gallsickness, which included various other diseases and conditions which the farmers commonly referred to as gallsickness. He stressed the importance of a blood smear examination for differential diagnosis to exclude other diseases and pointed out that no stock owner can afford to be satisfied with what he called a rough and ready diagnosis of gallsickness, when he may in reality have to guard against a serious epidemic disease.

It comes as no surprise to come across another paper in which the author tries to clear the confusion which existed in the use of the term "gallsickness". It was probably created by the Voortrekkers to signify the amount or consistence of bile in the gallbladder and ended as one of the most used and most abused terms amongst stock owners in South Africa[6].

The term 'gallsickness' is still used to describe anaplasmosis and, after it having been in use for more than 70 years, we might as well accept it in the South African situation and settle for "tick-borne gallsickness" as a good analogue for "anaplasmosis", to help differentiate between all the existing analogies.

Transmission of *A. marginale* and *A. centrale* to South African antelopes

The artificial transmission of *A. marginale* and *A. centrale* to species of South African antelopes has been demonstrated[5,3]. These experiments showed that *A. marginale* produced an active infection in the blesbok, the duiker and the black wildebeest, whereas *A. centrale* caused a latent infection only in the blesbok. Neitz[4] successfully infected blesbok with *Anaplasma ovis;* the parasites could be demonstrated microscopically and anaemia resulted from the infection. He was unable to transmit *A. ovis* from the blesbok to cattle. The important aspects of these experiments are:-

1. Cattle are susceptible to *A. marginale* and *A. centrale* only.
2. The blesbok is susceptible to all 3 species.
3. Cattle, sheep, goats, blesbok, black wildebeest and duiker are all susceptible to *A. marginale* infections.
4. The morphology and virulence of both *A. marginale* and *A. centrale* are not changed by passage through blesbok, duiker and sheep[5].

Practical implications of these findings

1. The fact that certain antelope are susceptible to *A. marginale* and *A. centrale* but refractive to *Babesia bigemina* and *Theileria mutans* indicates that these antelope can be utilized for separating these parasites, which usually occur together in South Africa. Neitz & du Toit[5] used this method to separate *T. mutans* from *A. centrale*. According to these authors, a mixture of these 2 parasites (together with *B. bigemina*) had been used for years in the vaccine to protect cattle against redwater and anaplasmosis. *T. mutans* was ignored in this process, because the infection which it produced was so mild that it did not seem to influence the reaction in South African cattle. However, a demand for South African *A. centrale* strain arose from several countries[5] and it seemed important to eliminate *T. mutans* from the donor animals as it was feared that the parasite might become virulent under certain conditions.
2. Perhaps, from an epizootiological point of view the most important implication is that certain antelope species may act as reservoirs for these pathogens. From their observations it was concluded that anaplasmosis was primarily a disease of antelopes and that bovines became infected when brought into areas where the disease occurred[5]. It is generally accepted in South Africa that antelope may play a role in the epizootiology of anaplasmosis, but their role has yet to be defined.

EARLY CONCEPTS ON THE EPIDEMIOLOGY OF ANAPLASMOSIS

Distribution of the disease

The presence of anaplasmosis was noted in the Transvaal, Natal and Eastern Cape Province[13]. Later reports indicate that anaplasmosis occurs over the greater part of South Africa and particularly in the hotter and more humid parts, but is less frequent in the Orange Free State[7].

Host parasite relationships

In most of his early reports Theiler mentioned that he had seen latent infections of anaplasmosis in blood smears of especially Transvaal cattle. He was also of the opinion that all cattle born in South Africa are susceptible to anaplasmosis, although the susceptibility of cattle varied greatly. He believed that adult native cattle, born and bred in infected areas, were non-susceptible, whereas imported cattle were highly susceptible to infection. According to him this was an obvious conclusion since calves become infected and recover easily.

Theiler[12 13] proved his point by placing newborn calves immediately in tick-free stables. The calves were injected with infected blood, and reacted to *B. bigemina* and *A. marginale*, but recovered easily. It was also believed that recovered animals acted as reservoirs of the parasite[10]

Differences between the various strains of *Anaplasma* were recognised[12 13] *A. centrale* demonstrably produced protective immunity against virulent *A. marginale* infections and was subsequently used as a vaccine against natural infections of *A. marginale*[14].

If calves become infected, they are immune. In infected veld they are constantly exposed to new infections and thus, it may happen that other diseases may cause a temporary breakdown of resistance to anaplasmosis[7].

Vectors

It was generally accepted at the time that blue ticks, *Boophilus decoloratus* and, to a lesser extent, *Rhipicephalus simus*, were the main vectors of anaplasmosis, as shown above.

Theiler[13] reported some interesting observations regarding the vectors. He found that by moving cattle from high altitudes to lower ones or from one part of the country to another, some cattle might still contract the disease. This he ascribed to cattle that did not become infected as calves, because, as he put it, "There are certain areas where the infection is present to a slight extent only." As far as the control of the disease is concerned Theiler[15] came to the interesting conclusion that: "The destruction of ticks will get to the root of the evil".

From this brief review, which covers the history of a certain era of anaplasmosis in South Africa, it is clear that, compared to our current knowledge of the disease approximately 70 years later, very little advance has been made that could lead to a better understanding and more effective control of the disease. All the important basic concepts of the epizootiology of anaplasmosis, are history.

CURRENT SITUATION IN SOUTH AFRICA

Vector distribution and disease incidence

At present, we have a reasonable understanding of the distribution of the important cattle ticks in South Africa[2]. We have always depended heavily on the presence of *B. decoloratus* and *B. microplus* to indicate enzootic and epizootic areas of anaplasmosis (Fig. 1), an approach which is based on Theiler's work, and also on evidence from reported outbreaks of anaplasmosis. It is clear from the chart in Fig. 1 that there is a good correlation between the blue tick distribution and the incidence of the disease.

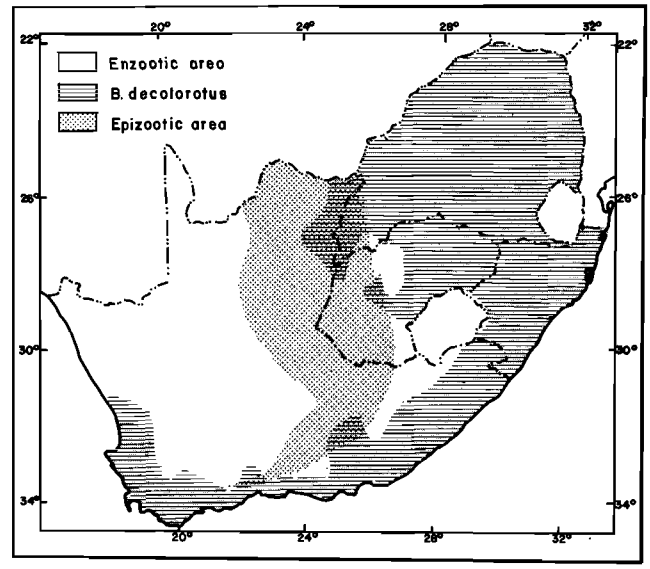

Fig. 1. The enzootic and epizootic areas of anaplasmosis in South Africa correlated with the distribution of *Boophilus decoloratus*.

Table 1 presents the results of attempts presently being made to identify other possible vectors of the disease.

Judging by the results obtained in our experiments, some of the tick species that occur in the epizootic areas from which blue ticks are considered to be absent, may play a role in the transmission of this disease. The enzootic areas as indicated in Fig. 1 include the Transvaal (except the far western part), Natal, North-eastern Orange Free State, South-eastern Cape and southern Cape coastal belt. The parts of the country which we believe to be epizootic anaplasmosis areas are the Western Transvaal, North-eastern Cape, eastern parts of the Karoo and the central and western Orange Free State.

Vaccine trials

Onderstepoort *A. centrale* vaccine has proved not to be as avirulent as was originally assumed (Bigalke, unpublished observations, 1971). Its use as a vaccine is attended by a certain amount of risk, especially in older animals, as indicated by the results obtained from this experiment.

For practical reasons, it was decided, in 1972, to alter the dose of the vaccine from 5 mℓ to 2 mℓ. This was made possible by using splenectomized cattle as donors which were bled only when showing a patent parasitaemia. Thus the quality of the infected blood could more easily be controlled. Prior to this some intact animals, used as donors, were periodically bled without showing a patent parasitaemia.

A second field experiment was carried out by Barrowman & Ferreira (unpublished data, 1977/78) to test the infectivity and pathogenicity of the "new" Onderstepoort *A. centrale* vaccine, some results of which are given in Table 3. Other important observations include:

1. Seven (16 %) of the 35 animals were treated when their haematocrits reached 20 %.
2. The first positives in the card agglutination (CA) test

Table 1: TRANSMISSION OF *A. MARGINALE* AND *A. CENTRALE* BY 5 DIFFERENT CATTLE TICKS

	Possible Modes of Transmission										
	Transstadial		Intrastadial			Transovarial					
Tick Species	L–N	N–A	L	N	A	A–L	A–N	A–A	A–LN	A–LNA	LN–LN
Boophilus decoloratus (1 Host)					1/1					0/4	
Boophilus microplus (1 Host)					1/1					0/1	
Rhipicephalus e. evertsi (2 Host)		0/3			3/4			0/2	0/2		0/1
Hyalomma m. rufipes (2 Host)					2/2			0/4	0/2		
Rhipicephalus simus (3 Host)					*2/2	0/1		0/1			

* Including the only successful transmission of *A. centrale*

Table 2: REACTIONS TO THE ONDERSTEPOORT *A. CENTRALE* VACCINE 5 mℓ GIVEN SUBCUTANEOUSLY (BIGALKE, UNPUBLISHED DATA, 1971)

Inoculum		Parasitaemia			Haematocrit			No. of animals survived
		Prepatent period/days	Max. %	Day Max.	Min %	% Drop	Day Min.	
A. centrale vaccine	$\bar{x}$	35	24,2	45	12	71,1	49	12/13
A. marginale challenge	$\bar{x}$		2,9	35,6	35,2	17,3		12/12
A. marginale controls	$\bar{x}$		14,28	31,4	17	59,34		5/5

Table 3: REACTIONS TO THE ONDERSTEPOORT *A. CENTRALE* VACCINE 2 mℓ GIVEN SUBCUTANEOUSLY (BARROWMAN & FERREIRA, UNPUBLISHED DATA, 1977/78)

Inoculum		Parasitaemia			Haematocrit			No. of animals survived
		Prepatent period/days	Max. %	Day Max.	Min. %	% Drop	Day Min.	
A. centrale vaccine	$\bar{x}$	44,8	(1–7 %)	52,5	30,6	17,2	52,6	35/35

ranged from 37–78 days with a mean value of 46 days.

3. Ten per cent false positive reactions were recorded with the CA test compared with the complement fixation (CF) test and blood smear examination.
4. No false negatives were recorded with the CA test.
5. Percentage agreement between CF and CA tests was 94 %.
6. Eight out of 43 animals remained negative after vaccination on blood smear examination, CA and CF tests.

Further studies are being undertaken by the author of this paper to compare the pathogenicity and immunogenicity of the *A. centrale* vaccine under laboratory conditions. Different field isolates of both *A. marginale* and one of *A. centrale* have been made from tick-transmitted infections and blood passage. The latter includes a mild strain of *A. marginale* from a buffalo *Syncerus caffer*.

ONDERSTEPOORT VACCINE

Present composition

The Onderstepoort tick-borne gallsickness vaccine has undergone no spectacular changes since it was first offered for sale in 1912. It is true that the same *A. centrale* strain isolated from a heifer in Aliwal North is still the only one being used today[15]. The vaccine production is based on maintaining *A. centrale* reservoirs which are bled as the demand for vaccine arises. Vaccination with *A. centrale* infected blood has been used in South Africa for many years to establish a state of premunity and confer resistance to subsequent *A. marginale* exposure[1]. Improvements to the quality control of the vaccine and various other safeguards have been made. To bring perspective to this particular aspect it should be stated that during all these years and up to the early 70's the *A. centrale* vaccine was regarded as very satisfactory. Subsequent improvements have resulted in the

following procedures being maintained under strict supervision.

Vaccine reservoirs

The majority of the present 32 donors are Hereford and Hereford Friesian crosses, all of which are splenectomized. These oxen and cows, the latter also used for breeding, were born, raised and maintained under tick-free conditions.

These animals are kept in isolation in tick-free stables which are kept scrupulously clean. The hay and straw used as fodder are steam sterilized in an autoclave at approximately 7 kg pressure for at least 2 hours to render them tick-free.

The animals are splenectomized just before they are weaned, that is, at approximately 6–7 months of age. The absence of the spleen increases their susceptibility to erythrocytic parasites, thus facilitating the exclusion of other adventitious blood parasitic infections.

The donor animals are inspected and their temperatures are recorded daily. Blood smears are examined for other blood parasitic infections every 14 days and immediately before bleeding. The parasitaemia of each animal is determined on a semi-quantitative basis and only animals which show a patent parasitaemia are bled. Individual animals showing the highest parasitaemia are recommended for use and are bled once every fortnight, if necessary. Approximately 1 ℓ/100 kg body mass is taken at a time. Animals which show signs of anaemia are rested until they are fully recovered.

All the cattle are vaccinated annually against anthrax, black quarter, Rift Valley fever, Wesselsbron disease and lumpy skin disease. They are also tested annually for freedom from contagious abortion, leptospirosis, tuberculosis and *Chlamydia* infection, and leucocyte counts are conducted to exclude leucosis.

Vaccine production

Infection of potential vaccine donors

These susceptible splenctomized animals are inoculated subcutaneously or intravenously with 5–20 mℓ of *A centrale* infected blood from any of the established *A. centrale* reservoirs showing a patent parasitaemia.

The ensuing *A. centrale* reaction is monitored by daily temperature, blood smear and haematocrit examinations. Oxytetracycline therapy at 10 mg/kg body mass given intravenously is subsequently applied when the haematocrit reaches the 20 % level. The animals recover and remain lifelong carriers of the parasite which fluctuate at a relatively low level and on occasion may even appear negative on blood smear examination. Not all animals become equally "good" donors, but some have remained in active production for as long as 15 years.

Vaccine

Blood is collected into sterile flasks containing an anticoagulant citrate dextrose (ACD) solution, which forms 20 % of the final volume on Friday mornings from donors showing a patent parasitaemia. Benzylpenicillin, 240 mg/ℓ (4×10^5 units) and streptomycin sulphate 200 mg/ℓ are added to the blood as preservatives.

Before the blood is pooled, each batch is tested for bacterial and fungal growth in order to trace individual animals that may harbour such infections.

The blood is bottled aseptically into appropriate vaccine containers, packed into insulated cartons and stored at ±4°C over the weekend before being dispatched on the Monday.

At present the demand for the vaccine is ±500 000 doses p.a. (See Table 4).

Tests on final containers

In view of the restricted shelf-life of the vaccine, tests for sterility and innocuity have to be done in retrospect.

Five final containers from each batch are selected at random and tested for bacterial and fungal sterility.

Three other tests are also carried out to identify possible viral contamination. The details of all these quality control tests are given in the Onderstepoort Manual of Procedures for the Quality Control of Onderstepoort vaccines, Vol I.

Use of the vaccine

The vaccine is perishable and has a limited shelf-life. The donors are bled on Fridays and the vaccine issued on Mondays. Since the vaccine should be used by the following Saturday, the expiry date is given as 6 days after issue. The vaccine should be refrigerated before use, but most not be frozen. The vaccine dose is 2 mℓ per animal regardless of size and is given by subcutaneous injection in the neck or chest.

The minimum number of parasites in each dose of vaccine is approximately 5×10^6 on the day the animals are bled.

Animals inoculated with the *A. centrale* vaccine normally react to a mild form of tick-borne gallsickness and recover spontaneously. However, severe reactions resembling those of typical anaplasmosis may develop, usually 5–9 weeks after inoculation. Such cases should be treated with recommended therapeutic agents at the first signs of ill health.

Recommendations

For optimum results it is advisable to immunize calves under the age of 6 months. Since older animals are more susceptible, the use of the vaccine is only indicated when such susceptible animals are to be introduced into anaplasmosis areas. It is also known that pregnant cows may abort and should preferably not be immunized before calving. In conclusion, it is still believed a single dose of the Onderstepoort *A. centrale* vaccine should give life-long protection against natural *A. marginale* infections.

Table 4: DEMAND FOR ONDERSTEPOORT *A. CENTRALE* VACCINE

Period	1971/2	1972/3	1973/4	1974/5	1975/6	1976/7	1977/8	1978/9
Total doses issued	367 729	381 057	542 923	667 844	867 360	708 556	643 660	496 530

GENERAL DISCUSSION

Most attention in the research of tick-borne diseases in South Africa is directed towards the improvement of existing vaccines and the ecology of the diseases and that of their vectors.

Our present knowledge of anaplasmosis and its application in our efforts to control the disease are briefly outlined[1]. The observations made at the beginning of this century make it clear that very little effort and therefore no significant contributions have since been made to improve the situation. Since 1977, however, it has been possible to apply the complement fixation as well as the card agglutination tests with reasonable success. These diagnostic aids can now be used to detect carrier animals and should be very useful in the envisaged epizootiological studies.

The results of the author's recent transmission studies with ticks have stimulated a fresh approach to vector studies in South Africa. To what extent ticks may become detached during any feeding stage in their life cycle only to re-attach to a different animal requires definition, since this behaviour could result in possible intrastadial transmission of anaplasmosis under natural conditions.

Adult male ticks are known to migrate on their hosts and they appear to be the most likely candidates to move from one animal to another, either by dropping off and climbing onto another animal or directly from one host to another.

The mechanical transmission of anaplasmosis by biting-flies should be investigated in South Africa. One attempt to establish such transmission with *Hippobosca rufipes* has so far failed but biting-flies can, it is believed, play a major role in mechanical transmission during an outbreak.

Enzootic areas of anaplasmosis in South Africa appear to have high vector activity and for the maintenance of a stable situation the obvious source of infection would be infected cattle. Wild ruminants may play a minor role as reservoirs of the infection but no field work has been done is South Africa to confirm this. At this stage of our investigation it seems that transovarial passage of the infection in various tick populations does not play a role in the maintenance of the infection. If this is the case in the field, it leaves premune adult cattle and young subclinical reactors (calves) and possible relapsing infections as the only sources of the parasite. Irrespective of the actual mode of transmission, it is still believed that adequate vector challenge will ensure early exposure of calves and result in the development of protective immunity.

Natural epizootic areas occur where none of the young calves are exposed to early infection because of the effect on vector survival resulting from geographical and climatic restrictions. Drastic fluctuations of these restrictions may result in the temporary settlement or elimination of vectors in these areas, with obvious consequences.

Human-induced epizootics may result within enzootic areas, if excessive tick control is practised. If vector ticks are eradicated, a farmer would, it seems, eventually breed susceptible stock. Under such conditions a lapse in the dipping programme, the possible introduction of carrier animals, the neglect by neighbours to dip their cattle, the presence of certain wild ruminants and migrating cattle, etc. could lead to serious disease outbreaks. As far as vector control is concerned, it is suggested that strategic dipping only should be applied in enzootic areas of anaplasmosis.

Chemotherapy could be applied to eliminate carriers of anaplasmosis within herds and such a practice could, together with aggressive vector control eventually eradicate the disease. It is accepted that such disease-free situations could possibly be achieved on a very small scale, but total eradication in this country appears to have a limited chance of success. One aspect, however, remains very clear and that is, if the arthropod transmission is not clearly understood, the disease cannot be effectively controlled under South African conditions.

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CONCLUDING REMARK

D.W. BROCKLESBY

As far as I am concerned these three days have passed very quickly indeed and this is usually a sign that you have enjoyed yourself. I have been very impressed with all the presentations. I have rarely been to a symposium at which we have the standard of presentations so noted. I have only got one criticism actually and that is the usual mistake people make about slides. I sat at the back one morning and I could see very few of them. If people would just make their slides a little bigger I think it would be a very great help. Sometimes at cricket matches and football matches in Britain they get some rather worn out old player to award a 'Player of the Match' prize and this is the most invidious thing of course and at a conference like this I think it is very invidious, but nevertheless I am going to do that. I am going to take it on myself to award my own 'Player of the Match'. There is no prize, just a remark and I don't think anyone will disagree with me. I would like to give this to Dr Lente van der Merwe for her presentation on Heartwater.

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