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TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINERE VERENIGING

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ADDRESS VOORDRAG

PAST-WORKERS ON TICK AND TICK-BORNE DISEASES IN SOUTHERN AFRICA

Paper read at the Seminar on Ticks and Tick-borne Diseases at ONDERSTEPOORT at a meeting of the Parasitological Society of Southern Africa on 15 January 1975.

GERTRUD THEILER*

PROLOGUE

There comes a time in everyone's career, when one stops in wonderment at how well one's predecessors knew what questions to ask and how they set about answering them. One's wonderment and admiration is increased upon studying their publications and upon finding out how they coped under their somewhat primitive conditions with very limited facilities, and yet produced outstanding results. As H. Watkins-Pitchford remarked in his presidential address to section D of the 1907 S.A. Association for the Advancement of Science meeting in Natal "Present workers need to be beholden to the good fundamental work done by their predecessors."

I have been requested to give a brief outline of our predecessors concerned with "Ticks and Tick-borne Diseases". A simple enough topic originally – but of such vast complications and ramifications these days that I must of necessity confine myself to detailing but a few aspects and merely outlining others.

Ticks have been with us since the beginning of time and probably, as the Revs. T. Arbousset and F. Daumas in 1846 stated – "torment the animals grievously". These two reverends were very observant of ticks, their association with cattle, the damage done by them, they recommended rubbing the part they attacked with tar or with tobacco juice; sometimes also ravens freed the cattle from them with their bills. The "small blue" is generally considered to be less dangerous than the other cattle ticks. Less observant was Ferdinand Krauss, a trained museum man, who toured from Cape Town to Port Elizabeth – between 1838 to 1840, who also noted the presence of ticks and who though otherwise observant apparently did not realize that there were several species nor that they were not the same as the Holzbock *(Iricinus)* of his native Germany. What other naturalist travelers, met or observed, is a subject wide-open for investigation by anyone who has the leisure to study his "Africana". Other travellers besides the two French missionaries also mention ticks in the Eastern Province – one even going as far as to mention how notorious Grahamstown was. So bad was the area that a Cattle Disease Commission was appointed at Grahamstown in 1877. Its findings give our first definite information on the question of heartwater and its suspected association by the local farmers with the introduction into the area, of the bont tick in the early 1830's. By the middle of the nineteenth century we find an awareness of the presence of several species of ticks, their association with man and his stock and the need to curb their activities and to prevent their dispersal.

Towards the end of the century C. Wiltshire, appointed in 1874 in Natal, and D. Hutcheon, appointed 1880 Cape, were well aware of the veterinary problems facing them but it was not until 1891 that a Colonial Bacteriological Institute was started in Grahamstown with Dr. A. Edington, a medical, in charge to serve both the medical and veterinary professions. In 1896 Theiler was appointed State Veterinarian and Government Bacteriologist to the Transvaal Government, and in 1897, H. Watkins-Pitchford was appointed as successor to C. Wiltshire as Principal Vet. Surgeon of the Colony of Natal. In the Western Province – where the wine and fruit orchards were being threatened by *Phylloxera* and fruit flies, an economic entomologist C.P. Lounsbury was appointed in 1895. So that at the turn of the century each province had somebody or organisation to whom the farmers could tell their woes and who could respond to the plaintive request voiced at an East London Farmer's Association meeting in 1899, inter alia, that the Government set up an experimental farm to study tick eradication – that dipping thanks be erected by the Government . . . and that "if only the Government would lend a helping hand the pest (heartwater and bont tick) could be mastered." The farmers were observant, the farmers were keen to own clean cattle – as well as clean sheep. Although as yet not 100% effective, the Scab Act, dating back to early days and the anti-scab dipping programme helped matters considerably. Before the turn of the century the veterinarian Duncan Hutcheon together with R.W. Dixon and J. Spreull in the Eastern Province did their best collecting information on, and experimenting with, heartwater and redwater. A. Edington also was busy with redwater. It was not, however, until the appointment of C.P. Lounsbury (publishing on ticks 1895 to 1909) and C.W. Mally 1901 to 1904 and Claude Fuller [who left for Natal in 1899 and falls out of the picture for ticks], that the study passed from the disease and its symptoms to the ticks, when Loundsbury, besides publishing on the fowl tampan (notorious in Grahamstown), did extensive studies on heartwater and redwater with notes on the biology and life cycles in the laboratory of *Amblyomma devium = (Amblyomma marmoreun)* and *Argas persicus* – published between 1895 and 1899. "Some bont females were so foolish as to bury themselves in the road dust." Claude Fuller, with previous experience in Australia, made a comparison between the Australian and South African blue-ticks and their relationships to redwater in N. America, Australia and possibly South Africa. In the meanwhile S. Wiltshire in Natal was doing his best to curb the spread of redwater, introduced in 1870 from the north via Zulu-

* Veterinary Research Institute, Onderstepoort

land, and which showed a high mortality in Chaka's days.

In that it had been established that Texas fever in America was carried by the blue tick, and in that in the minds of the Eastern Province farmers the bont tick was the cause of heartwater in their sheep, the Eastern Province farmers, especially those in the Albany district, commenced dipping, spraying or washing their stock in with apparently whatever they thought might be effective, from plant-pest sprays including an arsenical scrub – exterminator to washes for scab in sheep (thus by 1890 arsenic was already in use). W. Robertson 1901 even recommending internal sulphur with calves often being dipped in sheep tanks, or even in cattle tanks. Lounsbury in his 1902 – 03 Annual Report mentions that the tank on Campbell's Farm, Fort Beaufort district – "has proved too long and too deep for small stock" . . . "cattle run through at the rate of 10 to 12 a minute". "Dipping at 14-14 for nine months had no ill effect." Then came East Coast Fever (E.C.F.) via the railway route from Delagoa Bay to Komatipoort through the Eastern Transvaal to South Africa to bring ticks right to the forefront, the questions now being asked: where and what ticks were causing the trouble; what were the cheapest and readiest means of curbing their activities; what were their life cycles?

From now, on in this paper, I shall deal with the accumulating mass of information under separate headings.

TAXONOMY

C.P. Lounsbury plodded on with his experiments, under adverse conditions in Cape Town (he got his new buildings at Rosebank in 1903), he had all his tick identifications, as did A. Theiler, confirmed by L.G. Neumann of Toulouse. The first attempt at a publication on South African ticks was by C.W. Howard in 1908, Government Entomologist for Mocambique. Apart from L.G. Neumann's publications and Nuttall and Warburton's monographs, this was my "Bible" when I started taxonomic work. Howard was followed by W. Dönitz 1910 – "Die Zecken Süd Afrikas." Taxonomy as such, however, did not come into full flower until the appointment of the full time entomologist G.A.H. Bedford in 1912 at Onderstepoort, a taxonomist *par excellence*, who produced his final checklist-host-list in 1932. In the early 1940's F. Zumpt, working with museum material in Hamburg, attempted a classification of the Rhipicephalid adults, this museum work was followed up by work in the field in South Africa, during the 1950's to 1960's, when his interest in ticks began to wane, with a few odd publications which centred on disease carriers of wild mammals.

Other taxonomists in Southern Africa at this period are Gertrud Theiler 1941; J.A.T. Santos Dias, Mocambique 1947; V.A. Sousa Dias 1950, followed by F.M.H. Serrano 1963 in Angola; S.G. Wilson, Nyasaland *et seq.* 1936; R.W. Jack 1921 in Southern Rhodesia. In 1956 Hoogstraal updated tick taxonomy, giving clear descriptions and illustrations in his monumental *"African Ixodoidea Vol. I. Ticks of the Sudan."* Whilst not the "Be All & End All" of tick taxonomy this "Handbuch" is an essential tool to all African Workers and as such has already proved its worth, all subsequent identifications and descriptions of new species being based on it. Gertrud Theiler and collaborators Britha Robinson and Lois Salisbury revised the descriptions of South African ticks between 1941 and 1967.

GEOGRAPHICAL DISTRIBUTION

As tick taxonomy came to be stabilised, workers were in a position to attempt to piece together tick distribution pictures. The first one to realise the importance of knowing the distribution of ticks, especially of the disease vectors, was C.P. Lounsbury who in 1904 attempted a distribution map of *R. appendiculatus* on the little information available at the time. H. Sigwart in 1915 gave his findings for the northern parts of S.W. Africa; Bedford included in his 1932 check list what information he could gather from the literature.

It was not until 1937, with the advent of the Tick Survey alone and in conjunction with the Zoological Survey, mainly under the direction of A.D. Thomas, with his assistant Kolbe, that geographical distribution came into the picture in its own right. The material for the Tick Survey was collected by stock-inspectors and their staffs; the various museums sending in the parasites from their study material; the Plague Research Institute, under D.H.S. Davis, sending in parasites from their rabies – and other field rodent – control work. The veterinarians were concerned as to which tick occurred where, in relation to cattle movements and disease carriers. The findings of the Survey are summarised in the *"Ixodoidea. Parasites of Vertebrates in Africa South of the Sahara"* in 1962, which work also summarises the publications of Ethiopian workers, giving host lists, parasite lists, harmfulness and disease transmission and geographical distribution. Included are the findings of J.A.T. Santos Dias 1947 in Mocambique; V.A. Sousa Dias, 1950, Angola; S.G. Wilson, 1943 Nyasaland and East Africa; and material collected by K. Unsworth from Bechuanaland, and R.W. Jack, 1910 to 1942 in Southern Rhodesia. For South Africa, G. Theiler attempted to plot the individual tick distributions on the somewhat inadequate maps available. It was not until towards the end of the Survey that D.H.S. Davis' 1958 – *et seq.* standard grid maps, which allowed much more accurate plotting, became available. Also as from 1962 various gazetteers have been published allowing of a ready reference for known localities. These maps have been made use of by Jane Walker and collaborators in her publications on Ticks in Tanganyika 1967 and of Kenya 1974, including all the information garnered by her predecessor E.A. Lewis.

Of particular interest to me is Lounsbury's 1899 remark on *Ornithodorus savignyi* "it is said to be not uncommon in parts of Oudtshoorn" . . . "It is a wonderfully active but most repulsive creature". The nearest I got to establishing these records are my finding it at Allemanskraal in the Steytlerville-Noorsveld. These were the only ones sent in during the Survey years from this Karroo area.

BIONOMICS

Developmental periods and stages

Amongst the old hands, in connection with their tick transmission experiments, we find C.P. Lounsbury and A. Theiler breeding various species of ticks

and establishing their developmental periods: *A. hebraeum, H. aegyptium, evertsi, B. decoloratus* (also Koch 1898), *R. capensis, Rhipicephalus simus, A. devium* (=*A. marmoreum)* and *savignyi* and *A. persicus, Rhipicephalus appendiculatus, Rhipicephalus sanguineus; Haemaphysalis leachi;* Herbert Watkins-Pitchford in 1910 reared *R. appendiculatus* for his E.C.F. dipping programme. Lounsbury has many interesting remarks on his rearing difficulties as to temperature, humidity, and length of feeding periods. In his annual report of 1899 he gives much casual information of what is known about the biology of the ticks used in his experiments. It is to be regretted that of the old hands, besides Bedford, not one described the immature stages. The Zoological- and Tick-Survey material enabled me to rear an assortment from different parts of South Africa and to give descriptions of the immatures of many of our species. Some series are still awaiting intensive study, to establish the range of variations in adults within the F1 generation of a known female. We are still not sure of – shall we say – what range to allow for *R. simus* or *R. appendiculatus,* or of any other equally variable species. From this point of view the identifications of the Tick Survey may not be as accurate as one could wish; if it looked like A it was noted as A. Thus in the *R. appendiculatus* distribution some *Rhipicephalus pravus* were noted as *R. appendiculatus,* but in so far as the *pravus-appendiculatus* appeared in different climatic plant zones it was surmised that it might be a different species; as was proved upon rearing in the laboratory. The *R. capensis* map is such a jumble of environments that it is quite obvious that more than one species was noted as *R. capensis.* Hence we taxonomists have been asking ourselves whether, in some instances, we are not dealing with hybrids.

The material sent in for the Zoological Survey, together with the rearings and descriptions of the immatures of known ticks allowed a reasonably accurate picture to be drawn up as to the host preferences of all stages – essential information if one would want to know which alternate hosts could act as reservoirs of tick-borne diseases. D.R. Arthur and J.G.H. Londt have shown us how useful the Electron-scanning microscope can be in the identification of immature stages.

Field observations and Ecology

"2250 larvae have been counted at the top of a single blade of grass"; "by day and by night, through wind, rain and light-frost, they remain at their post", thus records C.P. Lounsbury in 1899 on *B. decoloratus* larvae. Grass was found to be still infested three ;months later. As to the bontpoots he noted that "we have indications that the species is well fitted to a dry climate; egg batches hatched in the office, where all other species died"; "the long drought of 1897 to 1899 in the midland districts did not appear to affect its numbers".

In connection with control measures during 1904 to 1906 for E.C.F. A. Theiler and S. Stockman 1904 set up experiments to show how long an area will remain infected with E.C.F.; in 1905 they studied the influence of cold on ticks and on *Piroplasm parvum.* They established that after 18 months the area could be considered clear, when tick-free, clean or recovered cattle could be re-introduced; for tests had shown recovered cattle to be *Theileria parva* free, hence if there were a breakdown in a herd the outbreak was assumed to be due to the introduction of infected ticks. It was not until recently that Neitz showed that E.C.F. recovered cattle were not sterile but carried a very low parasitism – allowing but a small chance of a tick becoming infected and for a recrudescence after intervals varying from 2 to 15 years – hence the adoption of the slaughter out policy for the complete eradication of the disease, if not of *R. appendiculatus.*

The next serious studies on the fate of tick larvae under various field conditions, including the annual flooding of the Kafue Flats, were done at Mazabuka, Northern Rhodesia by W.J. Gray during 1957 to 1961. Gray explored the possibilities of using radio-isotope-tagged ticks; followed by Mary Kraft in the Eastern Province; whilst S. Stampa worked on *Ixodes rubicundus* mainly in the Karroo areas of New Bethesda. In 1962 A.D. Thomas published a short note on the behaviour of ticks in the Northern Transvaal. Maureen Baker still has some unpublished observations on haystacks and their larval burdens on a farm in Natal.

Environmental Resistance

The only laboratory studies on environmental influences on ticks are: (1) R. Gothe 1967, whose findings on the Boophilids tally with their geographical distribution. Field studies are being carried out, in that notorious tick centre Grahamstown, J.G.H. Londt and G.B. Whitehead have established preferred heights on the vegetation for the larvae of various species of ticks e.g. 40 to 90 cms. for *A. hebraeum* – but like observant farmers they fall down on the deductions they draw i.e. that each larval species climbs to a height most suitable for the size of the animal to be infested, forgetting that even the largest bovine has its feet on the ground upwards, or lies recumbent in the shade of a tree; forgetting that the smallest bird comes to rest, feeds and even nests well above the grass at ground level – thus *A. hebraeum* larvae at 40 – 90 cm are situated "just fine" for these intermediate hosts. The height to which larvae will migrate upwards, to my mind, is *not* associated with host-finding, but with the search for a favourable climate and with larval resistance, or lack thereof, to micro-environmental conditions. In general their findings on the ecology of the tick larvae studied, confirm the tick distribution pictures as established by the Tick Survey. (2) W.O. Neitz, F. Boughton and H.S. Walters 1971 on the Life Cycle of *I. rubicundus* under different controlled laboratory conditions.

Host-parasite Relationships and Host-preferences

Although Lounsbury, Theiler and Bedford were able to establish some host preferences it was not until the results of the Tick- and Zoological-Survey came to hand that a picture could be drawn up, when G. Theiler states "In reviewing the host preferences of the African ticks it is clear that few are limited in their distribution by lack of a suitable host."

Within a given climatic/vegetation zone it could be expected that each separate species of tick, during its seasonal activity period, will be present at the same time on the various species of herbivore hosts; the numbers present on any one type of host being dependent on population densities and on host availability. J. MacLeod and Kate Jooste 1971 working in

Rhodesia, however, show that this is not so. They find that, within a given uniform region, tick infestation patterns and levels show quite a range of variations within the seasonal period of activity for each species of tick. Thus in the Sebungwe-Gokwe area *R. appendiculatus* for the suids has its peak in December to February; for the antelopes in March to May; for *R. pravus* there is a curious alternation of the seasons in the infestation level of the antelopes: cold – hot – rainy – post-rainy; this alternation is not evident in the suids, the bush-pig having a distinct peak in the cold season, lowest in the hot season (is this habitat associated incidences?). This dissimilarity in seasonal host-infestations needs to be taken into account when we are trying to establish the seasonal incidence of a tick. As yet no one in Southern Africa has worked on host-size, population-size, tick-load and standard range of the host. We need to remember also that many host parasite records may only reflect an environmental host encounter relationship, incidental, and not necessarily a host preference, e.g. many argasids feed readily on laboratory hosts, but succumb soon after repletion.

Seasonal Incidence and predilection Sites

The results of much of the work done on the seasonal incidence and or predilection sites of ticks on domestic animals undoubtedly remain on the files of various workers and institutions. A couple of publications come to mind: Kate Jooste 1960 on *R. appendiculatus* in Rhodesia; Maureen Baker and F.B.W. du Casse 1967 to 1968 on the main economic species of livestock in Natal.

TICK-BORNE DISEASES

Bacterial, viral, and protozoal

The standard starting point for this topic is Theobald Smith and F.L. Kilborne 1892 to 1893 on Texas-fever and cattleticks in the U.S.A. In 1898 R. Koch established *B. decoloratus* as the carrier of redwater in South Africa, as was suspected by the farmers in Natal, where redwater was already known in 1870 being introduced from the North. By 1893 the disease had spread to the Cape. G. Lichtenheld and W. Kolle 1908 report on it in S.W.A. In general tick transmission work in S. Africa had to wait until after the Anglo Boer War. C.P. Lounsbury at the Cape, however, was able to publish on *A. hebraeum* and heartwater in 1900 and on *H. leachi* and biliary fever in dogs in 1901. In 1903 he was seconded for a few months to the Transvaal, working at Theiler's E.C.F. Station in the Elands River Valley. Finalising his work at his new laboratory at Rosebank, he was able to show *R. appendiculatus* to be the carrier of *T. parva*, confirmed by Koch 1903 in Rhodesia (under *R. sanguineus*). Lounsbury is probably the only worker who could ever report: "the experimental heifer died at a waterhole, probably after being overpowered by a crocodile". From now on, one can imagine the excitement both at Rosebank and at Daspoort when either Lounsbury or Theiler could establish a new vector, or could confirm the finding of others, so that at the time of Union, the vectors for redwater, E.C.F., Benign E.C.F., heartwater, gallsickness, spirilosis in cattle, biliary fever in dogs and in equines, were well-known. At this period much transmission work was being done in Europe e.g. E. Brumpt, H. Laveran and collaborators in France, at Liverpool R. Newstead, S.R. Christopher and collaborators on Central and W. African species; in London G.H.F. Nuttall, H.B. Fantham and Annie Porter [who came to S. Africa to work on bilharzia in 1913]; Theiler and Nuttall exchanging ticks, E. Hindle, G.C. Purvis ex Grahamstown laboratory; in Algiers Etienne and Edmund Sergent, also in consultation with Theiler; laterly A. Donatien and F. Lestoquard and L.M. Parrot, F.C. Wellman in Angola *(O. moubata);* David and Lady Bruce alone or together with J.E. Dutton, J.L. Todd and J.W.B. Hannington in Uganda. In 1909 Theiler visited Bruce's set up, after which they collaborated on *R. appendiculatus* & Amakebe disease; G. Lichtenheld 1911 in German East Africa. During and after the 1914-18 war there seems to be a gap in tick transmission work. In the mid-twenties, with the introduction of splenectomy it got a new lease of life. The work done during the period 1920 to the present day, I leave to my colleague W.O. Neitz to deal with on another day. Apart from Neitz' various summaries on protozoa and tick-borne diseases B.A. Matson 1967 gives a good bibliography 1897 to 1966 on Theileriosis.

Tick paralysis and tick Toxicosis

Tick paralysis first described from the Eastern Province, was ascribed to *Ixodes pilosus* by J.B. Hellier 1892, C.W. Mally 1904 and by J.D. Borthwick and J. Spreull 1905, before Neumann's *I. rubicundus* description had reached South Africa; now popularly known as the Winter-Paralysis Tick. In 1938 R. Clark showed *R. evertsi* to be responsible for paralysis in the O.F.S. – (spring lamb-paralysis). Work by W.O. Neitz has shown that only some strains are capable of transmitting the condition. Other Onderstepoort records for tick paralysis are *Hyalomma truncatum* in many parts of South Africa; *Rhipicentor nuttalli* and *R. tricuspis* in Rhodesia; paralysis in human beings in South Africa has been ascribed to *R. simus,* F. Zumpt and D. Glajchen 1950; *H. truncatum* L.D. Erasmus 1953, Pretoria, and A. Swanepoel 1959 Piquetberg.

Tampan paralysis in fowls was first recorded by C.P. Lounsbury 1904; *inter alia* he recommends cyanide fumigation for *"A. persicus"* control; and J.D.W. Coles 1947. R. Gothe, who collected his *A. persicus* species during his 1966 stay in South Africa is actively studying fowl-paralysis in Germany, with the assistance of a physiologist and a nerve specialist. W.O. Neitz 1963 ties tick paralysis up with *Tick Toxicosis,* including sweating sickness caused by some strains of *H. truncatum;* as also *R. appendiculatus* or brown-tick toxicosis, caused by a leucocytotropic toxin, as worked out by W.O. Neitz and A.D. Thomas 1958. S. Stampa and S. Stampa & R. du Toit 1958 – 1959 published on the symptoms and field observations of winter-paralysis in the Karroo. The latest publication to hand, dealing with tick paralysis and its possible explanation, is that of J.D. Gregson 1973 "Tick paralysis an appraisal of natural and experimental data", Canada Dept. Agriculture, Monograph No. 9. Notes on the environment of *I. rubicundus* are made by S. Stampa 1958 – 1959 and by J.L. v.d. Walt 1964. Control measures are discussed by J.D. Botherwick 1905, S. van Rensburg & E. Silcock 1929, W.M. McHardy 1950 – 1958; and S. Stampa & R. du Toit. The present recommendation is a

walk-through dipping tank with a suitable residual acaricide; two dippings at an interval of five weeks offers complete protection during the period of winter activity of the adult. Way back Mally 1904 reports that: "Among the native herdsman, leaving the ticks on a sheep is considered a sure sign of madness".

HISTOLOGY AND CYTOGENETICS

To date the only South African publication on the internal anatomy and histology of ticks is that of Mary W. Till 1959 – 1961, on *R. appendiculatus*. With S.R. Christopher's 1906 – 1912 work as a bsis F.C. Welman 1907 investigates some bodies found in *O. moubata* fed on *Filaria perstans*. R. Gonder 1910 attempted to describe the life cycle of *T. parva*, as did W. Steck in 1928; G.H.F. Nuttall, H.B. Fantham and Annie Porter attempted to cultivate *T. parva* in 1909. During 1926 E.V. Cowdry spent some time at Onderstepoort working on *Rickettsia ruminantium* as also on a group of micro-organisms hereditarily transmitted in ticks. In 1938 E. Reichenow published on *T. parva* in *R. appendiculatus*. Undoubtedly for lack of knowledge of the normal cellular changes in ticks of the various phases of the organs during their three different stages, and of the cells of the digestive tract during digestion, cells undergoing a normal phase change may have been mistaken as an introduced element and interpreted as a phase change of a protozoan. How much work has been done on the internal histology of ticks at Onderstepoort, I know not, much possibly that has never yet been published? Neitz in 1959 introduced tissue culture to study the development of his protozoa, doing away with the necessity of knowing the histology of the ticks. In 1964 H.M. Martin, S.F. Barnett & B.C. Vidler in Kenya publish on the cyclic development and longevity of *T. parva* in *R. appendiculatus*. In 1966 R. Gothe & C.J. Howell had the courage to branch out into cytogenetic studies on the chromosomes of *O. savignyi* the sand tampan while C.J. Howell's other works on Karyotypes remain to be published.

DISEASE PREVENTION BY TICK CONTROL

Disease prevention by pest control both in the plant and animal world is a long established practice; the more we learn about a pest the more specific can the treatment become, whether by chemicals, by predators, parasites or by alteration of habitats or by stock movements.

Chemical control

In his history of dipping P.M. Bekker 1960 covers the field of chemical control fairly adequately; regrettably his coverage of the early days is poor. Much of S. Stockman (left South Africa in 1904) and A. Theiler's work in the Transvaal being overlooked. Also, quite obviously many of Lounsbury's official reports were overlooked e.g. his 1899 Cape tests reporting results to G.V.O. Duncan Hutcheon at Cottesbrook but more especially his E.C.F. recommendations when after sundry dipping experiments he could state *in 1904* that "Fortunately an effective remedy for the tick is now known to be at hand in arsenical dips". This statement was based on experiments in which he showed that stock suffered no ill effects at 14 days dipping intervals carried on over nine months. He, however, had to concede that the 14 – 14 interval did not kill *R. appendiculatus*, which, according to his rearing experiments, could attach and fall off during the 14 days' grace. In his 1903 – 1904 Annual Report Theiler records that dipping (= spraying) for E.C.F. at 4 or 5 days interval was not practical in an irritating and poisonous bath. He also advises against erection of common tanks because of the risk of clean cattle getting infected at the tank or *en route*, quite apparently realizing that arsenic had but a short residual effect. Much of this early literature is bedevilled by the somewhat loose use of the words "dip" and "dipping", when a chemical wash or sponging or spraying were implied. Thus S. Stockman's "dipping control stations" were in reality "spraying stations". H. Watkins-Pitchford in Natal was successful in solving the arsenic-burn difficulty by testing its efficiency at lower concentrations, to be applied at shorter intervals. Though the words "dips and dipping" are used somewhat loosely, there is no question but that tanks were in use before Baines erected his at Nels' Rust, Natal; Hellier 1891, Douglas 1896 e.g. Campbell's Tank, Fort Beaufort district and private veterinarian Buck's tank 1901 at Kimberly, so that Bekker's statement that Baines' was the first cattle dipping tank in South Africa and that he initiated dipping in arsenical washes, is incorrect.

Where as H. Watkins-Pitchfords' short interval pilot-tests had given good results they have not stood the test of extensive field experience, when during the 1920's there was a breakdown in the ECF control measures, drawing attention to the necessity not only of knowing the wash's chemical composition and strength but also of studying the introduced extraneous matter with its arsenic-resistant bacterial-flora – so ably studied by H.H. Green – and later the appearance of the arsenic-resistant tick in the Eastern Province, as recorded by P.M. Bekker *et al*, during 1939 – 1942.

The advent of D.D.T., B.H.C., etc. introduced teams of fresh workers, including J.R. Malan, not veterinarians but chemists, the new chemicals being tested under the aegis of: Onderstepoort, Grahamstown led by A.B.M. Whitnall and his compatriot G.B. Whitehead, and East London L.C. Blomefield, McHardy, G.E. Thompson and J.A.F. Baker. Many of these workers are still active, some are with us here today prepared to tell us about their latest findings. Some, e.g. H. Graf, J.A. Thorburn, have contributed their mite and like their predecessors in turn are beginning to become legendary figures. When we come to the effect of arsenic on cattle it is a case of "fools rush in where angels fear to tread" e.g. the entomologist C.P. Lounsbury 1900 – 1905, the Field Veterinarian S.T. Amos 1909, the laboratory veterinarians H. Watkins-Pitchford 1911 and D.T. Mitchell 1918 reported on this aspect; whereas the biochemist-physiologist H.H. Green and his collaborator C.D. Dijkman 1918 confine themselves to the elimination of arsenic in the urine; and in 1938 the physiologist-pharmacologist Douw G. Steyn and his chemical collaborator describe the symptoms of arsenical poisoning in cattle and fail to mention what happens to the arsenic in the body. At this time much work was being done on safe dosages of arsenic relative to wireworm control in sheep. I stand open to correction: no one in South Africa has worked on the fate of arsenic in stock after Douw G. Steyn's 1938 publication?

Predators and parasites

No systematic work has been done in South Africa. A brief scanning of the literature gives these findings:

Mites: W.J. Gray 1956, Northern Rhodesia, *Tyroglyphid Mites* destroying *R. evertsi* eggs.

Reduvid bugs: F.C. Wellman 1906, Benguella, *Phonergates bicoloripes* destroying live *O. moubata.*

Ants: A. Theiler 1911 states that ants destroy many of the ticks which drop from their hosts, but records no definite observations.

W.J. Gray, 1957, Northern Rhodesia, ants, *Anopholepis custodiena,* records four female ticks to be killed within 40 minutes; workers of *Pheiodole liengmei* were found feeding on dead *R. evertsi.*

Flies: W.J. Gray in Southern Rhodesia noted one instance which is suggestive of an Asilid attack on an adult tick.

Mice: Sergent 1945, Algeria: *H. mauritanicum,* hibernating in kraal walls, will emerge in spring provided mice can not get at them in the cracks.

Birds: According to my recent scanning of the literature the following have been recorded as having ticks in their digestive tract: *Bubulcus ibis,* Cattle Egret; Domestic fowl; *Numida meleagris,* Guinea fowl; *Afrotis africa,* Black Korhaan; *Streptopelia c. capicola,* the Cape Turtle Dove; *Tockus erythrorhynchus rufirostris,* the Red-billed Hornbill; *Tockus flavirostris,* the Yellow-billed Hornbill; *Calandrella starki,* Stark's lark; *Acridotherus tristis,* the Indian Mynah; *Buphagus* spp., the Red and Yellow-billed oxpeckers; *Corvus albus,* the pied Crow; *Corvus ruficollis,* Sahara Crow; according to the behaviour of starlings overseas we could expect to find that our starlings also pick off the ocassional tick.

The consensus of opinion is that present day dipping practices have reduced the numbers of oxpeckers throughout Africa and that they are gradually being confined to National Parks and Game Reserves. They certainly are not as plentiful around the Onderstepoort neighbourhood as they were in 1909. The Cattle Egret, however, has increased enormously in numbers since then, and may replace the oxpecker as the chief tick-predator on birds. The local egrets, however, are apparently being affected, not so much by dipped cattle, as by the insecticides used on the local market gardens and on other cash crops.

Parasites: The literature on South African tick parasites is but meagre.

Fungi: As yet I have seen no positive record of moulds killing ticks. O.G.H. Fiedler's work on *Beauveria thuringensis* remains unpublished.

Chalcids – Encyrtids: Although *Hunterellus hookeri* occurs naturally in South Africa no work on its possible use, after Lounsbury's 1908 effort has as yet been published. During the 1960's E.M. Nevill, working with material collected off wild hares at Kaalplaas, tried to rear it but was unsuccessful. In 1953 O.G.H. Fiedler, otherwise concerned with the chemical control of blow flies, described *Hunterellus theilerae* off *H. truncatum* on a wild hare; it has since been recorded in *H. truncatum* off a wild hare in the Transvaal, G. Theiler and Fiedler 1953 and by Hoofstraal and M.N. Kaiser 1958 – 1961 in the Cairo area in *Hyalomma rufipes* off migrant *Oenanthe* species. It is of interest to note that amongst the vast number of ticks sent in for the Tick Survey, there was not one infected nymph, so that one can assume that *Hunterellus* is not playing any rôle in curbing our tick explosion. That R.A. Cooley in 1934 should have found as many as he did on wild hares in South Africa, amazes me.

Pasture management, etc.

But little work has been done on pasture rotation alone or in its several modified forms. A. Theiler 1904 as also H. Watkins-Pitchford after him, in his E.C.F. experiments, after temperaturing, moved recovered cattle after "dipping" onto well fenced clean lands. Other than this I know of no other publications on this question, of change or alternation of pastures apart from S. Stampa, 1959, in his *I. rubicundus* and tick-paralysis control work; as J.C. Bonsma, 1944, has tackled the question of natural and acquired resistance of cattle to the attachment of ticks. Lounsbury 1904 studied the resistance of Persian and cross-bred persian sheep to heartwater. As yet no work has been done on sterilisation of male ticks and their liberation.

G.B. Whitehead, 1973, is actively studying the protective mechanisms of the tick to acaricidis.

TECHNIQUES

Collecting and rearing, etc.

I have not gone into the contributors to these aspects of tick work starting with Lounsbury and Theiler; and Koch who had trouble rearing his ticks. The information is well covered in current text books.

Mention must, however, be made of E.M. Nevill's 1964 paper on the use of CO_2 to attract *O. savignyi* and C.J. Howell's 1966 bright idea of using *Pilocarpine hydrochloride* as a pharmacological stimulant for the collection of its salivary gland secretions. In South Africa it would seem that *O. savignyi* has replaced the almost universally used *O. moubata* as the Argasid laboratory "guinea pig". C.J. Howell 1969 still has to publish his final findings on his analysis of its saliva. D.R. Osterhoff and R. Gothe 1966 published on the use of red-cell antigens for timing the breakdown of erythorocytes during digestion in *O. savignyi.*

EPILOGUE

This hastily thrown together review I hope has succeeded in showing how well our predecessors answered their questions and how in turn the answers raised new and more complex questions, so that by January 1975 the field is wide open to newcomers who are welcome to work in one of many fields, be he a veterinarian, a parasitologist, physiologist, neurologist, biochemist, physicist or geneticist. As G.A. Walton (*O. moubata* – complex in East Africa) now Professor at Cork University, Ireland, expressed it in his 1975 New Year's greetings to me "The graduates here are doing fine work on ticks and on *I. ricinus* here, however, it is sophisticated and advanced stuff on amino-acids, pheronomes, electrophoretic identity of their arthropod and invertebrate predators and enzymes. The literature forms a solid platform and for that we have to thank you for your help."

J'ai dit

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BOOK REVIEW

RESENSIE

THE IXODID TICKS OF KENYA

JANE B. WALKER

Commonwealth Institute of Entomology, London, 1974
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Publ. Price £5 Sterling

This review assembles and analyses the available information, published and otherwise on the hosts, distribution and disease transmission of the 73 species of Ixodid ticks that have so far been recorded in Kenya. The author is to be commended for the inclusion of so much unpublished data gathered from reports and records not otherwise accessible to the reader.

The book gives a detailed description of the country, its climate, vegetation and livestock populations, all well illustrated by maps and figures. A considerable section is devoted to host-parasite lists and to the transmission of tick-borne diseases of animals and man in Kenya, the latter presented in tabular format.

The distributions of the tick species are well-illustrated in 33 separate maps and their host relationships, distribution and ecology discussed in the text. References to useful re-descriptions with, if necessary, notes on the identification of each species are supplied.

This review is well-written and the numerous maps, all black and white, are throughly annotated and form an integral part of the text. It can be recommended as a valuable acquisition to all those interested in the ecology and distribution of parasites of man and animals.

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REVIEW OORSIG

RECENT ADVANCES IN SEMEN PRESERVATION

W.J. NEVILLE*

SUMMARY

While the horizons for cryobiology may be bright and granted, tremendous strides have been made in the area of artificial insemination (A I), it is evident that only dairy cattle A I with frozen semen has achieved industrial application. Beef cattle A I has been hampered by the lack of suitable techniques for oestrous control. However, with the advent of prostaglandin F2α, it may now be possible to make the genetic progress which has been accomplished through A I in dairy cattle. Genetic progress through A I is possible in all farm species but the overriding influence has to be economic.

At this juncture it appears that A I with boar, ram and goat semen hardly warrants the excessive costs entailed in freezing and distribution. Frozen boar and ram semen can become realistic goals if and when sires of superior genetic merit become available through progeny and performance testing. It is also possible that frozen boar semen will find application in specific pathogen free herds or perhaps in international trading of genetic material. When oestrous control is developed to such an extent in the species mentioned, that precise timing of ovulation is possible, then A I with frozen semen can be justified. It is also imperative that semen processing and freezing techniques be standardized to enable breeders to achieve repeatable results.

Frozen dog and stallion semen have little to offer in the way of practical application in light of the restrictions imposed by breeding organizations, to warrant intensive investigation. However, if it can be accepted that the integrity of breeders can be policed with parentage and other controls, then frozen dog and stallion semen will have far reaching benefits for the avid animal lover and sportsfan. In light of the apparent problems surrounding poultry semen freezing, it seems that greater emphasis will have to be placed on maintaining viability for short periods of time in ambient temperature diluents. Even though frozen human semen is a fascinating subject for the students of eugenics, and may find application in a "Brave New World", yet it is unlikely that man has evolved to the stage where he can accept the concommitant implications.

INTRODUCTION

We are on the threshold of a new era of biological research. The gradual shift in emphasis from the marvels of atomic and radiological aspects of physical science to the exciting horizons of the molecular and biochemical facets of biological science is gaining momentum. Perhaps the most important advance in the last decade has been the concept of genetic coding, which depends upon the salient and interrelated roles of DNA and RNA. The implications of first understanding and then controlling the dissemination of genetic information on a cellular level are boundless. Emphasis on increasing our basic knowledge of most aspects of reproductive physiology is a necessary prerequisite for complete exploration of genetic findings. Within the framework of this emerging emphasis, banking of spermatozoa and ova in suspended animation for perhaps time immemorial, will play an intriguing role if not a vital one.

The first scientific research in semen preservation dates back to the discoveries of Spallanzani[86] who observed that stallion spermatozoa frozen in the snow was not necessarily dead but held in suspended animation. A new landmark in semen preservation was established by the remarkable discovery that glycerol afforded protection to spermatozoa during the hazards of freezing at ultralow temperatures[65]. This discovery set the stage for the development of cryobiological techniques for the long-term preservation of a variety of cell types. In particular, the fortuitous discovery of the cryoprotective properties of glycerol was the necessary impetus for the development of the routine low temperature preservation of bovine semen, which in turn has been the key to genetic improvement of dairy cattle throughout the world.

* Animal and Dairy Science Research Institute, Private Bag X2, Irene, 1675.

WORLD REVIEW OF FROZEN SEMEN A QUESTIONNAIRE[37]

World usage of bovine frozen semen

As can be observed from Table 1, the number of countries where frozen semen was used during 1970 for routine artificial insemination work in cattle was 22, while it was only three in 1962. The widespread usage of frozen semen is dependent on a number of factors:

1. Frozen semen is used under circumstances where artificial insemination (A I) has been highly extended and well recognized for a number of years.
2. Conception rate is not inferior to that of fresh liquid semen.
3. Liquid nitrogen is inexpensive and readily available.
4. Semen containers and other instruments can be readily obtained.
5. Existence of a situation where the use of frozen semen will result in increased economic gains.

World usage of frozen semen of farm animals other than cattle

Frozen semen of farm animals such as sheep, goats, pigs and horses has not been applied to any great extent in the world. However, limited use for frozen goat semen has been reported from Greece since 1967. Frozen horse semen has been used for practical A I in

Table 1: COUNTRIES WHERE THE NUMBER OF A.I. COWS WITH FROZEN SEMEN WAS ABOVE 400 THOUSAND ANNUALLY OR ITS USING RATE WAS ABOVE 50%. (BASED ON STATISTICS OF 1970)

Name of Country	Total No. A.I. Cows (A)	No. of A.I. Cows with Frozen Semen (B)	Using rate of Frozen Semen (B/A) %
Ireland	1 033 045	406 000	39,3
Great Britain	2 239 010	1 784 913	79,3
Norway	416 766	416 733	100,0
Sweden	597 580	597 580	100,0
Finland	809 625	809 625	100,0
Denmark	1 388 219	500 000	36 –
F.R. Germany	3 781 423	2 657 795	70,3
Belgium	525 306	444 022	84,5
France	7 312 133	–	99 –
Portugal	70 120	52 590	75,0
Switzerland	321 506	–	100–
Austria	500 988	494 144	98,6
Czechoslovakia	1 869 569	1 062 724	56,9
Yugoslavia	970 000	700 000	79 –
South Africa	81 834	81 834	100,0
Israel	113 486	113 486	100,0
Japan	1 817 285	1 121 337	61,7
Canada	1 094 966	1 094 966	100,0
U.S.A.	8 578 778	8 578 778	100,0
Mexico	192 200	191 904	99,8
Brazil	62 188	53 612	86,2
Australia	149 000	102 000	68,5

Table 2: EFFECT OF 16 DIFFERENT COMBINATIONS OF FREEZING AND THAWING RATES ON SPERMATOZOA SURVIVAL IN STRAWS STORED AT – 196°C FOR 2 WEEKS[1]

Method	Mean cooling rate from + 5 to – 60°C		Post-thaw motility when thawing in water bath at				
	+ 5 to – 15°C	–15 to–60°C	5°C	35°C	75°C	95°C	Mean
	(°C/min.)	(°C/min.)	(%)	(%)	(%)	(%)	(%)
Controlled forced vapour, goblets (BF –3 –2)							
I. 5°C/min. from + 5 to – 15°C 10°C/min. from – 15 to 60°C	6	12	35	40	44	44	40,7
II. 10°C/min. from + 5 to – 15°C 20°C/min. from – 15 to – 60°C	11	23	35	41	43	44	40,6
Rapid forced vapour, goblets (BF –3 –2)	20	76	35	39	43	43	39,9
Static vapour (–180°C) single straws on racks (LR –250)	95	53	37	41	44	43	41,1
Mean			37,5	40,0	43,4	43,2	

Table 3: EFFECT OF VARIOUS COOLING RATES AND THAWING TEMPERATURES ON SPERMATOZOA SURVIVAL IN STRAWS FROZEN SINGLY IN STATIC VAPOUR (8 EJACULATES)[2]

Starting vapour temperature	Mean cooling rate from + 5 to – 15°C		Motility after thawing in water bath at	
	+ 5 to – 15°C	–15 to – 60°C	5°C	75°C
	(°C/min.)	(°C/min.)	(%)	(%)
– 90°C	32	23	28,6[a]*	47,4[c]
– 120°C	43	25	31,9[b]	46,2[c]
– 150°C	50	27	32,6[b]	47,1[c]
– 180°C	82	43	33,9[b]	47,8[c]

a, b, c means followed by different superscript letters are different at the 5% level of probability

* indicates significance at the 1% level of probability

Table 4: EFFECT OF DIFFERENT METHODS OF FREEZING STRAWS UPON SPERMATOZOA SURVIVAL AFTER STORAGE AT – 196°C FOR 3 WEEKS (20 EJACULATES)[2]

Method	Total No. closes per freeze	Mean cooling rate from + 5 to – 60°C		Post-thaw motility after strange for	
		+ 5 to – 15°C	– 15 to – 60°C	1 day	3 weeks
		(°C/min.)	(°C/min.)	(%)	(%)
A. Rapid forced vapour, goblets (BF–3–2)	1 920	17	65	30,8	32,0[a]
B. Controlled forced vapour, goblets (BF–3–2)	1 920	12	23	35,8	34,0[a,b] *
C. Static vapour, (–170°C) goblets on rack (LR–250)	240	14	21	33,2	31,2[a]
D. Static vapour (–180°C) single straws on racks (LR–250)	240	108	62	37,6	37,2[b]
E. Ampules, slow cooling (BF–3–2)	576	1	4	38,0	36,4[b]

a, b means followed by different superscript letters are different at the 1% level of probability.

* indicate statistical difference between methods B and D at the 5% level of probability.

a number of districts in Japan. The recent success with frozen boar semen in the U.S.A. and Germany may find application in transport of "superior genes" between countries. However, despite the vast amount of research effort that has been devoted to frozen semen in farm animals other than cattle, little has accrued by way of application.

OUTLINE OF PROCEDURES FOR DEEP FREEZING OF BOVINE SEMEN

Semen storage packages

Three kinds of semen packages are currently in vogue, namely, the straw, glass ampoule and pellet. Currently the vinyl straw is the most popular throughout the world, primarily, because of the greater storage capacity obtained with straws. In addition to storage, the improved freezability of semen in straws as compared to ampoules is considerable (Tables 2 - 4); The pellet system of semen freezing and storage is practised in Finland with good success. The main advantages of freezing and storage in pellets is the tremendous number of inseminations that can be stored in a unit volume of liquid nitrogen. The main disadvantage arises with identification of pellets from individual sires. The 0,5 ml straw is currently the most popular package in use throughout the world. However, considerable attention is being placed on the 0,25 ml "mini-straw" in an effort to reduce storage expenses still further.

Diluents (extenders) for frozen semen

There are probably as many diluents for frozen semen as there are investigators in this field. However, the egg yolk buffered solution, egg yolk solution to which sugars and several other substances are added or egg yolk-milk are currently the most popular. The major reason for the variety of semen diluents available is possibly due to the fact that semen being a biological fluid has certain physiological requirements for survival during storage and hence a wide variety of buffering systems, cryoprotective agents and energy sources are conducive to survival during freezing and storage.

Glycerol concentration and equilibration time

Glycerol is the most widely used cryoprotective agent available and efforts to find a replacement (because of its contraceptive action in pigs and poultry)[48] have been to no avail.

The cryoprotective activity of glycerol is difficult to explain — numerous theories have been postulated as to why glycerol has to be incorporated in the semen extender for the protection of spermatozoa against freeze damage[42] These authors in their study concerning the mode of action of glycerol in protecting tissues against freezing injury, reported a reduction in the amount of ice formed in the tissues frozen after immersion in glycerol. They concluded that the protective action of glycerol was due to a combination of factors — easy cell penetration, low toxicity, effective water-binding capacity and a low eutectic point.

The concentration of glycerol used in the semen diluent varies with the individual A I centre and the kind of diluent used. The majority of cattle A I centres use between 5 and 7% (v/v) in egg yolk solution, 3,5 to

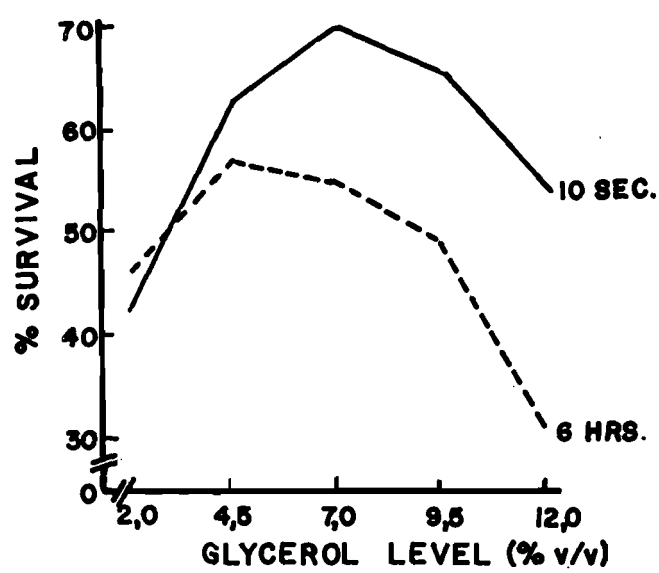

Fig. 1 : Effects of glycerol level and exposure time on post thaw motility[8]

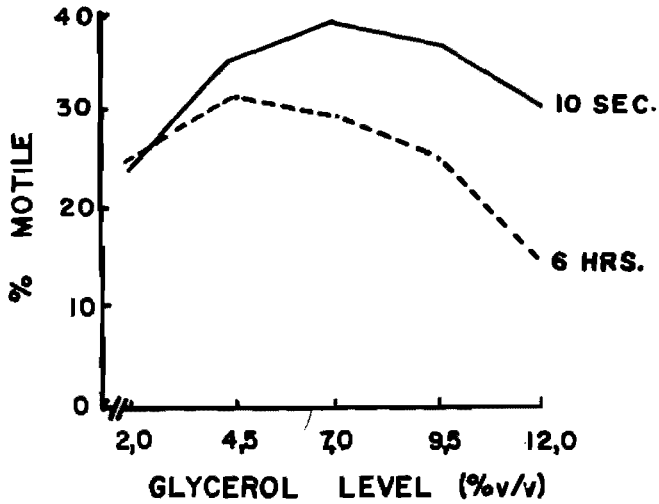

Fig. 2 : Effects of glycerol level and exposure time on percentage survival[8]

4,5% in pellet method and 10% in milk solution[57]. Glycerol equilibration time also varies with individual A I centres and in some circumstances equilibration time may even vary from individual to individual.. In general equilibration time varies between 4 and 18 hours. However, the tendency is to shorten glycerol equilibration time as it would appear that the longer semen is maintained in the non-frozen state outside the body the greater the loss in viability (Figs. 1 & 2). This is possibly due to a depletion of the endogenous energy reserves of the sperm cell[44]. Dilution of semen is generally made in a two step fashion except in the case of the pellet where glycerol is added in a second dilution at 4°C to 5°C. One step dilution is made with diluent containing glycerol at about 30°C in the pellet method. In Kenya, where the straw method is adopted, glycerol is added at 32°C when its concentration is 3% and at 4°C when it is 7%, respectively. In Brazil, one step dilution to the final volume is made at 5°C.

Freezing procedure

Freezing and thawing curves for bull and boar semen are presented in Figs. 3 and 4.

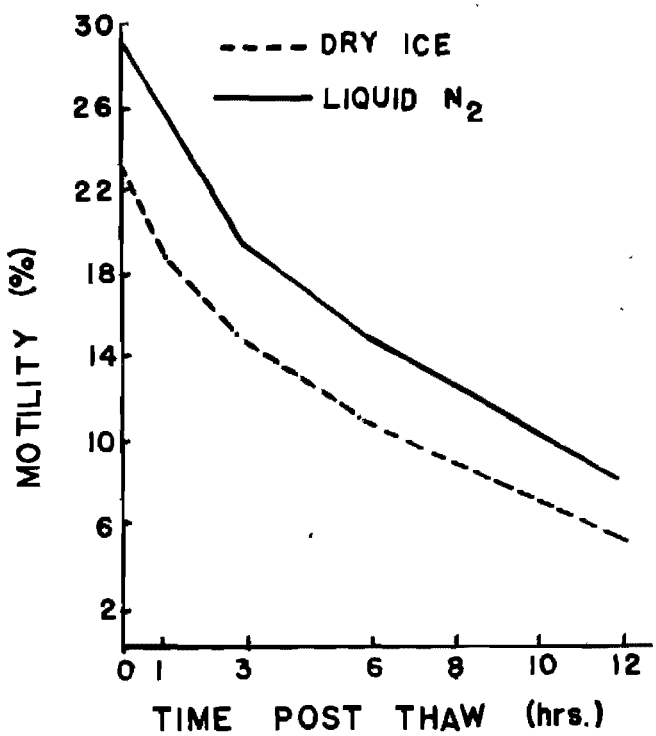

Fig. 4 : The effect of time post thaw at 5°C on per cent motility of bovine spermatozoa.[64]

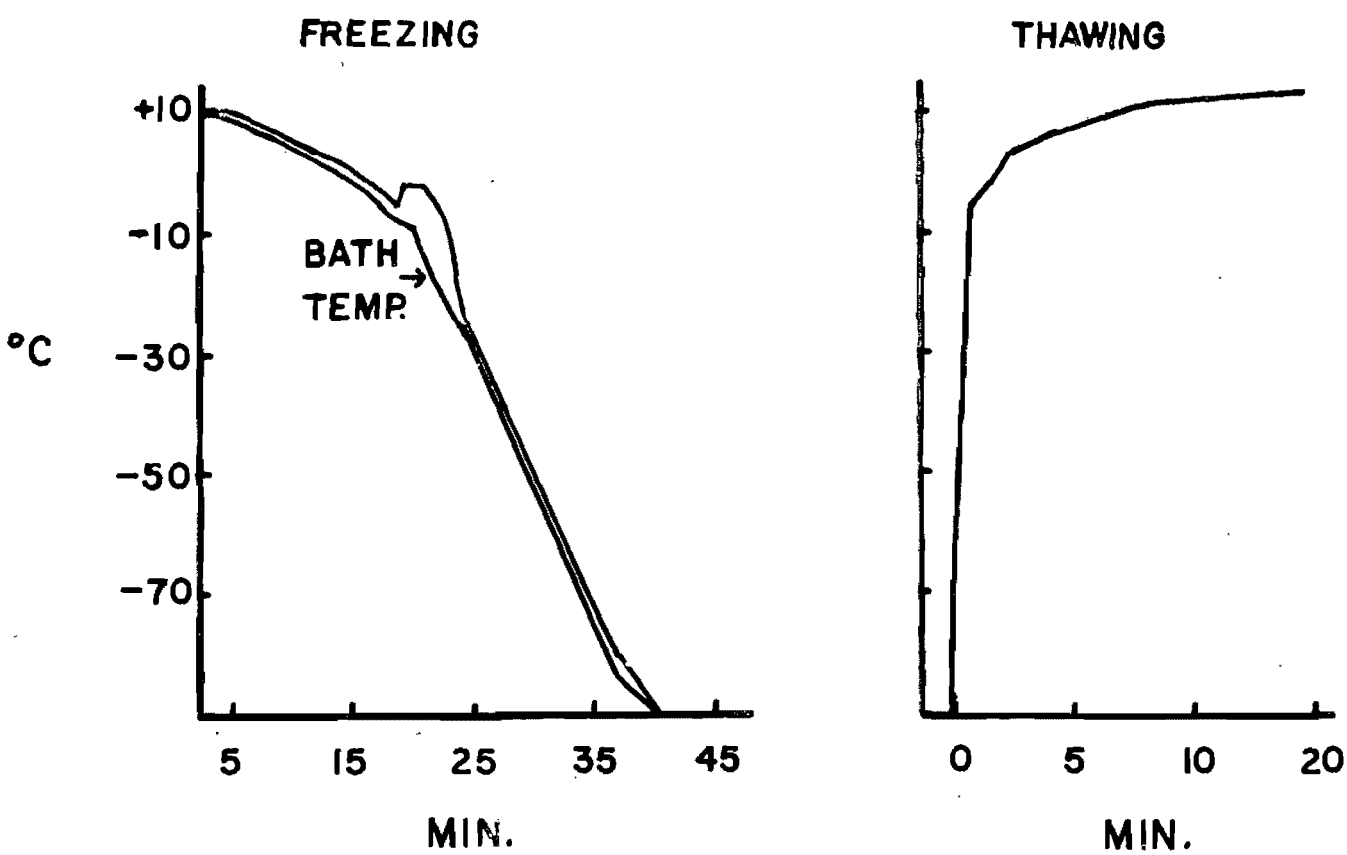

Fig. 3: The freezing and thawing curves of boar semen.

In the pellet method freezing is accomplished by dropping approximately 0,2 ml of diluted semen into small indentations made on dry ice. All of the countries where straws or glass ampoules are used employ the rapid freezing method whereby semen is frozen from 4°C to -80°C within several minutes in liquid nitrogen vapour. It has been established that rapid cooling is a necessary prerequisite for obtaining good recovery after freezing and thawing[62 69].

Temperature and duration of storage of frozen semen

European countries and most other countries, except the U.S.A. and Canada, had used the temperature of about -79°C using dry ice-alcohol or a mechanical freezer up to 1962. The U.S.A. and Canada had already employed liquid nitrogen (-196°C) as the storage medium and had unequivocally demonstrated that semen frozen in liquid nitrogen in some instances resulted in a 10% higher conception rate than semen stored in dry ice[57]. However, the application of liquid nitrogen was delayed for several years due to the difficulty of supply and the relatively high cost. Today all of the countries that use frozen semen use liquid nitrogen as the storage medium. Most of the developing countries of the world are still hampered by the cost and availability of liquid nitrogen and consequently frozen semen is not being used as extensively as it might be, despite the tremendous genetic progress that can occrue from its use.

The limit of the preservation period during which time frozen semen will still retain its fertilizing ability has not been firmly established. The longest reported storage period of frozen semen with successful conception is 12 years[45]. More recently it has been reported

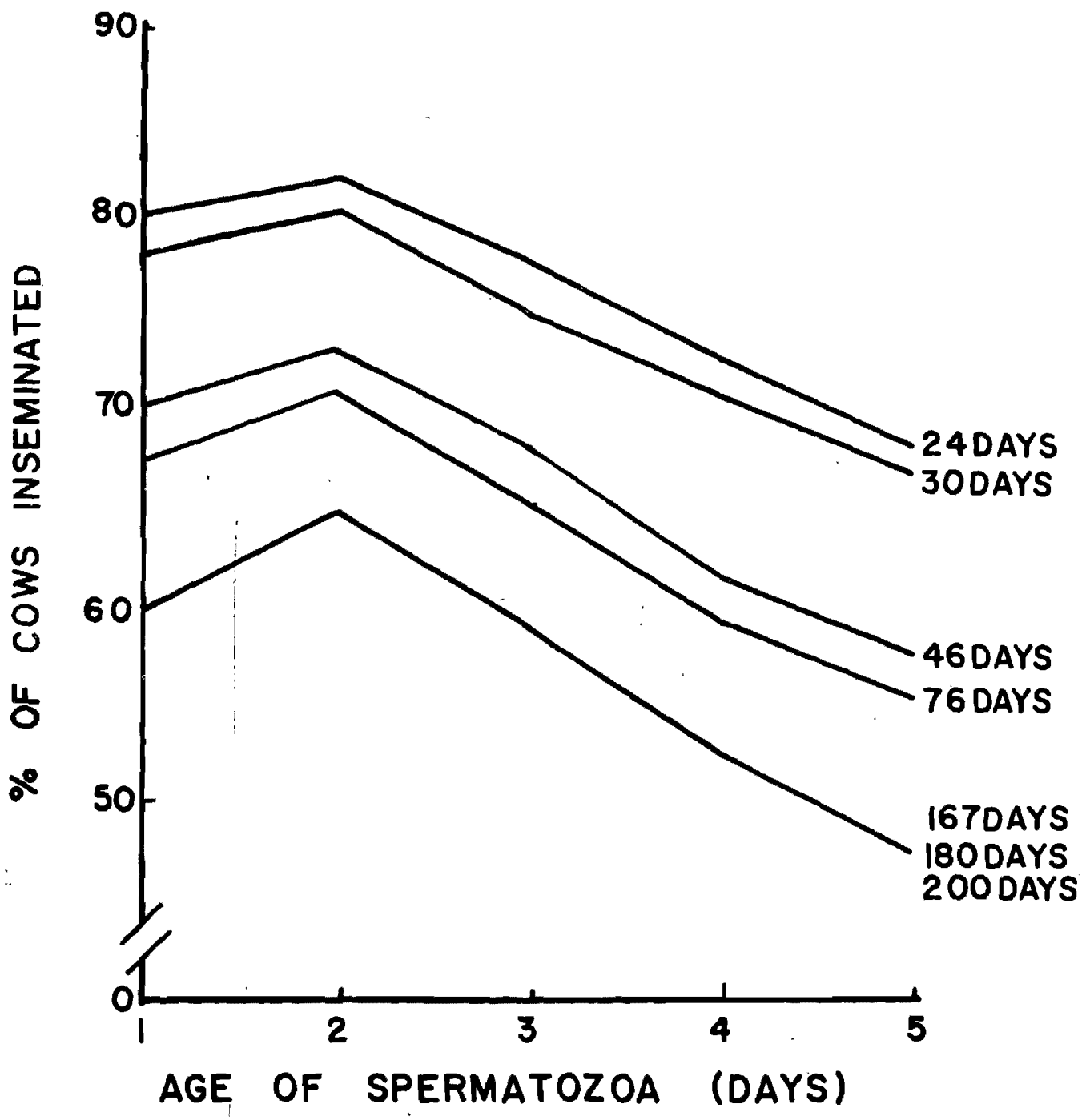

Fig. 5 : Effect of spermatozoan aging and time after insemination on fertility of cattle[57].

that semen stored for up to 2 years from bulls did not result in reduced fertility[20]. The average 150-180 day non-return rate (NR) for 17820 inseminations from semen stored up to one year was 66,9% NR and for 3222 inseminations for semen stored up to 2 years was 68,8% NR. However, another study presented evidence for a reduction in fertility as spermatozoa were aged *in vitro*[81]. (Fig. 5).

Thawing temperature and interval from thawing to insemination

A thawing temperature of 4°C to 5°C is employed in the U.S.A., Japan, Mexico, Republic of China, Phillipines and Iran, whereas Australia, New Zealand and F.R. Germany use a thawing temperature of 15°C to 20°C[57]. In most countries semen is thawed at between 30°C and 40°C suggesting a beneficial effect from rapid thawing. It is common practice to inseminate the semen as early as possible after thawing as the duration of survival after thawing is greatly reduced in comparison with non-frozen semen[47 64].

Number of spermatozoa inseminated

The number of spermatozoa inseminated is an important factor for the effective use of bulls especially in the practical application of A I with frozen semen. Generally the number of spermatozoa is approximately 15 million (30 to 50 times dilution) live spermatozoa[87] (Figs. 6 - 9). It is increased up to 30 million

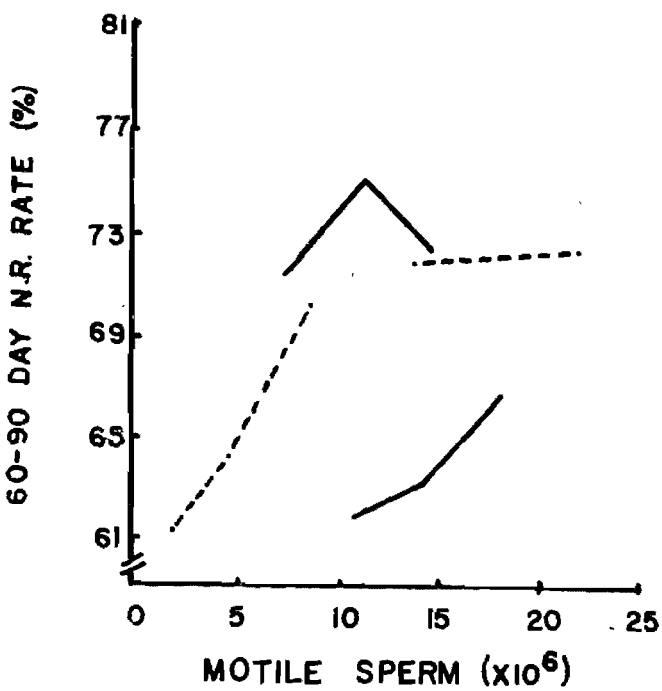

Fig. 6: Effect of sperm numbers upon the non-return rate for semen stored in liquid nitrogen. (Summary of four experiments)[87]

for bulls of questionable fertility. However, the tendency is to reduce the number of spermatozoa inseminated . The Dairy Board in New Zealand[72] reported that currently an insemination dose of 2,5 million total spermatozoa in an ambient temperature diluent is used nation-wide with a success rate comparable to the fertility levels reported for most countries. In comparing 2,5 million spermatozoa with 0,5 million per inseminate dose in a caprogen diluent

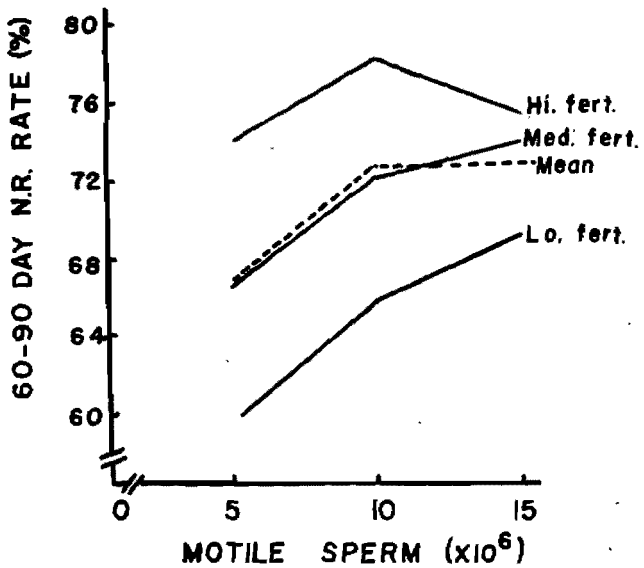

Fig. 7 : Non-return rate as affected by motile spermatozoa concentration and fertility level of Holstein bulls[87].

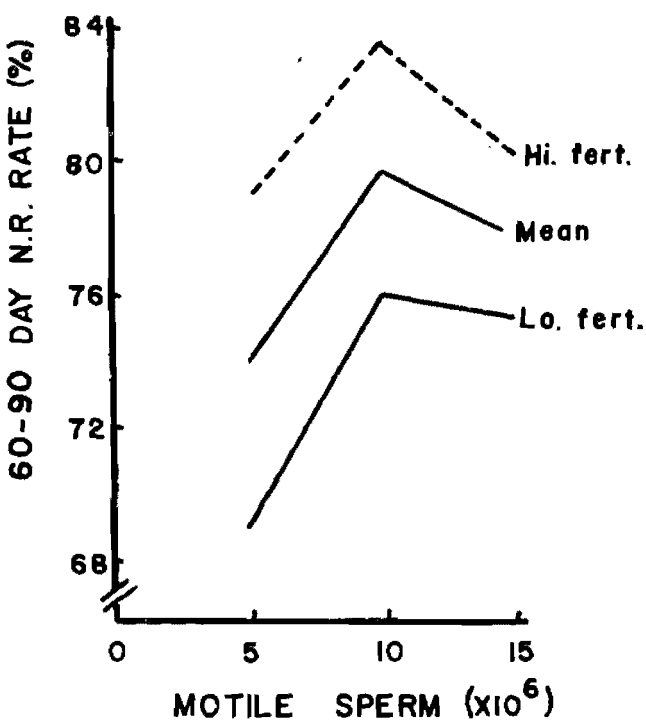

Fig. 8: Non-return rate of dairy cows as affected by motile spermatozoa concentration and fertility level of semen from Angus and Hereford bulls[87].

with an egg yolk concentration of 5%, it was demonstrated that the difference between conception rates was not significant[83] By using 2,5 million spermatozoa per insemination up to 60 000 inseminations can be obtained from the top proven bulls in New Zealand.

RATIONALE FOR SEMEN PRESERVATION

The key to freezing spermatozoa is to prevent the formation of hazardous ice crystals within the sperm cell. This is accomplished by using glycerol. In addition a semen extender must have the following properties[53]:

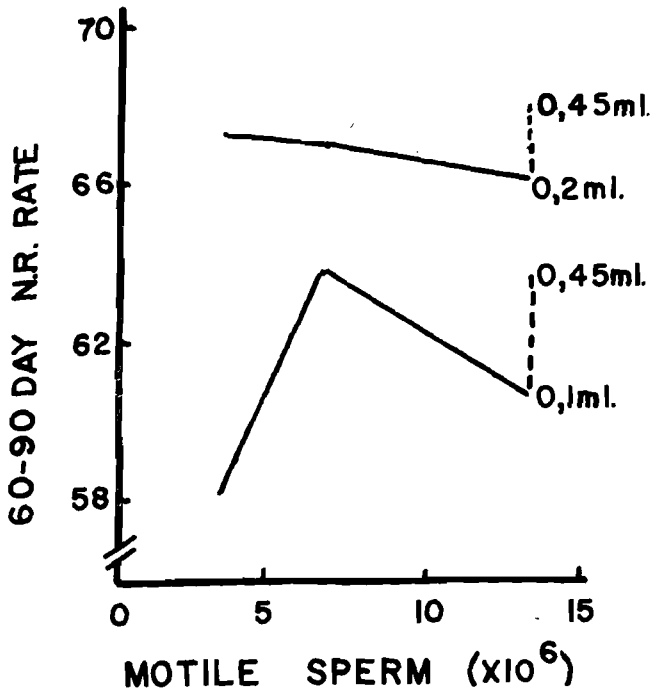

Fig. 9 : 11 Effect of volume and sperm numbers upon the non-return rate[87].

1. Isotonic with seminal plasma - this is approximately equivalent to an osmolarity of 320 milliosmols[19].
2. It should contain an adequate buffering system to buffer H± ions which result from glycolysis - a pH of 6,8 to 7,2 is adequate for most species[14].
3. It should contain constituents which protect the spermatozoa from cold shock - lipoproteins, which are abundant in egg yolk are beneficial[60].
4. It should contain certain nutrients for sperm cell metobolism – the primary need being energy for motility. Fructose, glucose sorbitol and a host of other sugars and sugar alcohols are readily metabolized by spermatozoa[44] .
5. It should be free from pathogenic bacteria and other harmful substances. Penicillin (1 000 IU of penicillin per ml) and dihydrostreptomycin (1 000 ug of dihydrostreptomycin per ml) are the most widely used antibiotics[17].

EFFECT OF SOME ZWITTER-ION BUFFERS ON THE FREEZING AND STORAGE OF SPERMATOZOA

Until recently few substances suitable for use as zwitter-ion buffers between pk6 and 8 had been available[21]. Several zwitter-ion buffers were investigated and it was reported that sperm stored in these buffers were more motile than those diluted with phosphate or citrate[31]. N-Tris (hydrozymethyl) methyl-2-amino ethane sulfonic acid (TES) with Tris (hydroxymethyl) amino methane (TRIS) as the titration base was the most satisfactory buffering system for diluting bull spermatozoa for freezing. These findings were confirmed with boar spermatozoa, where N-2 hydroxyethyl piperazine-N-2 ethane sulfonic acid (HEPES) and TES were superior buffers for maintaining motility and preventing glutamic oxaloacetic transaminase release from the sperm cell[14].

It has been demonstrated that all of the hydrogen ion buffers investigated were superior to conventional buffers (TRIS-HCL, phosphate and citrate) for maintaining motility of turkey semen. The lower ionic strength buffers, namely N-Tris (Hydroxymethyl) methyl glycine (TRICINE), HEPES and TES were superior for freezing, whereas TRICINE and HEPES were not as good for storage of turkey semen at 23°C. A combination of TES +NaOH and KOH (TES NaK) was superior to all other buffers in maintaining motility. TRIS was unsuitable for use as a titration base for hydrogen ion buffers for dilution of turkey semen[10].

From the foregoing it would appear that these zwitter ionic buffers have an important role to play in preserving semen from species such as the boar, ram, goat, stallion and turkey where it has been shown than conventional extenders such as milk and egg yolk-citrate extenders have been somewhat unsatisfactory. It should be borne in mind that species and individuals vary considerably as to the type of diluent which affords protection during freezing and therefore it is imperative when freezing semen from indiduals that more than one freezing medium and/or freezing regime is employed. This is particularly important in the case of the "problem bull" where a change in diluent composition or freezing procedure may readily ameliorate the problem.

CATTLE SEMEN PRESERVATION

Cognizant of the tremendous genetic progress which can be achieved by the use of superior bulls through young sire progeny testing and performance testing, we still see limited use for A I in general and for frozen semen in particular.

In a very comprehensive review on the factors affecting the utilization of frozen bovine semen for maximum reproductive efficiency, it has been reported that most of the fertility problems associated with frozen semen were due to improper handling of semen by the inseminator (technician) and not by the personnel freezing the semen[64]. The services of a skilled technician are essential to the success of an A I programme. Howeer, technicians vary in their ability to obtain and maintain satisfactory conception rates[46]. A difference of up to 15,7 percentage points in 60 to 90 day NR between six "low" and six "high" rated technicians in three breeding associations has been recorded[24]. The evidence also indicated that more cows returned to service after 30 to 60 days when bred by the "low" category technicians. It was also reported that when inseminators knew that they were being investigated the results obtained were far superior to the conception rates prior and subsequent to the trials[25]. The epitome inseminator influence was reached when inseminators who were tested for efficiency with buffer only without any spermatozoa obtained a 60 - 90 day NR to first service of 18%. This ludicrous situation was probably also in part due to the fact that non-returns to service are very unreliable as an indicator of a bull's fertility. The role of the A I technician appears to be of vital importance to the successful implementation of A I in cattle. However, it must be recognized that fertility is the resultant culmination of a number of complex and diverse phenomena. The importance of factors, such as semen handling, freezing and thawing procedures as well as timing of insemination and site of semen deposition cannot be minimised[25 64].

Despite the vast amount of research that has been devoted to preservation of cattle semen little has ac-

crued in the way of new discoveries or breakthroughs. On reviewing the literature for the past 20 years it is apparent that a multiplicity of factors influence fertility of semen and most trials report a beneficial effect of between 2 to 5% in fertility from a host of semen additives and newer procedures. However, on close scrutinization of the overall situation it becomes readily apparent that the average 60 - 90 day non-returns to first service is between 60 and 70%, which has not changed drastically since the inception of frozen semen[57].

The most striking advances have been the switches to liquid nitrogen as the storage medium and the use of the vinyl straw as the storage package. It has also been demonstrated that the use of caprogen diluents at ambient temperatures appears to allow for maximum use of superior sires[93]. This diluent also appears to be beneficial in the long term preservation of boar semen at ambient temperatures[34]. Another encouraging prospect for the cattle A I industry is the finding that the additon ofa or β amylase to the freezing medium resulted in significantly greater fertility results than the controls[32]. It was further demonstrated that glucuronidase also had a beneficial effect on fertility when added to bovine semen. The rationale behind these semen additives is that spermatozoa are capacitated *in vitro* and hence do not need long exposure to the uterus or oviducts to attain the capacity to fertilize[32]. These findings may have exciting possibilities for the *Bos indicus* breeds where allegedly the heat cycle is much shorter than in *Bos taurus* breeds[1]. However, further research is needed to establish if factors other than amylase or β glycuronidase exert a beneficial effect.

BOAR SEMEN PRESERVATION

The widespread use of A I in swine has been hampered primarily due to lack of suitable methods for the long term preservation of boar semen. The physical, physico-chemical and chemical characteristics of boar semen vary considerably from that of other species[26].

The boar is unique in that it produces a very large volume of semen over a long ejaculation period. During ejaculation semen is emitted with varying sperm cell concentrations and different types of fluids. It would appear that the chemistry of boar semen may be of great importance in understanding the physiological mechanisms underlying preservation of the sperm cell.

To date, no completely reliable method of preserving boar semen for prolonged periods of time is available that can justify the economic outlay involved. Certain extenders will maintain motility of liquid boar semen for up to 12 days. Fertilizing capacity is, however, greatly reduced after 24 hours. Preservation of liquid boar semen has been the subject of extensive research[35 36 92]. Illinois variable temperature diluent and caproic acid - citrate-egg yolk diluents as recently modified, appear to be beneficial to boar semen preservation for up to 3 days as evinced by motility and fertility[48 54]. If boar semen could be preserved at ambient temperature for up to 3 days it would greatly facilitate A I in pigs and would permit maximum utilization of superior sires at a minimal cost.

Research on frozen boar semen has been the subject of intensified investigation over the past few years. Graham[29] reviewed the literature on frozen boar semen and also reported on what would appear to be the first authenticated fertility result with frozen boar semen.

This rationale for freezing would appear to be related to the TES-TRIS buffer combination without glycerol as a cryoprotective agent. Several studies have shown that glycerol is a contraceptive agent in pigs when inseminated after storage of sperm for some time in glycerol — containing media[48 49 66]. However, more recently it has been demonstrated that if the glycerol is added immediately prior to insemination or freezing the contraceptive action of glycerol is not as apparent[15 48].

Satisfactory fertility using up to 4% glycerol in the freezing medium even though the glycerol was added to the semen some 1½ h prior to freezing, has been obtained[80]. In light of the reported contraceptive action of glycerol by some authors and the inconsistency of results obtained, it would appear that the rationale for freezing boar semen still remains unclear[29 30 80]. Further research is warranted as to the exact mechanism involved in freezing of boar semen, when one considers that fertility has been obtained in a number of laboratories in recent years using techniques which resulted in no fertility previously. Frozen boar semen is still an unrealistic goal until some repeatable rationale for freezing is proposed.

The most satisfactory fertility results with frozen boar semen have been reported following surgical in-

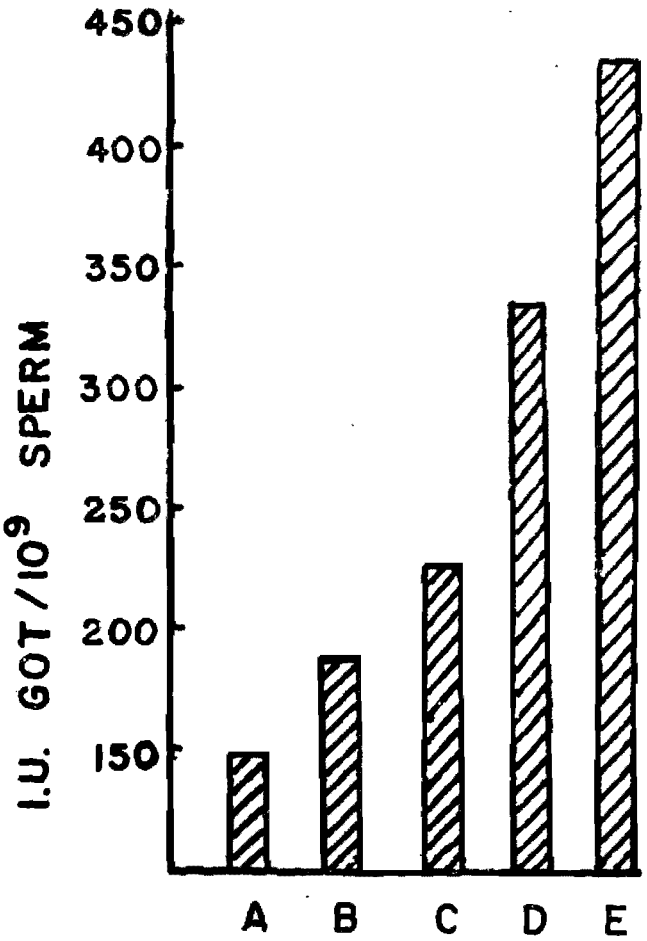

Fig. 10 : An example of enzyme analysis in monitoring cellular damage showing release of enzyme from cell after. A. Minimal cell damage. B. Cooling. C. Equilibration. D. Slow Freezing. E. Maximal cell damage[27].

semination into the ovi-ducts[67]. When semen was inseminated via the cervix or into the upper uterine horns no fertility resulted. These findings indicate that even in the presence of glycerol, frozen boar semen will result in satisfactory fertilization if transported to the site of fertilization. Therefore it appears that even though spermatozoa in frozen boar semen have the ability to move after thawing they do not have the necessary viability to move to the site of fertilization. It has been reported that glumatic oxaloacetic transaminase (GOT) and hyaluronidase

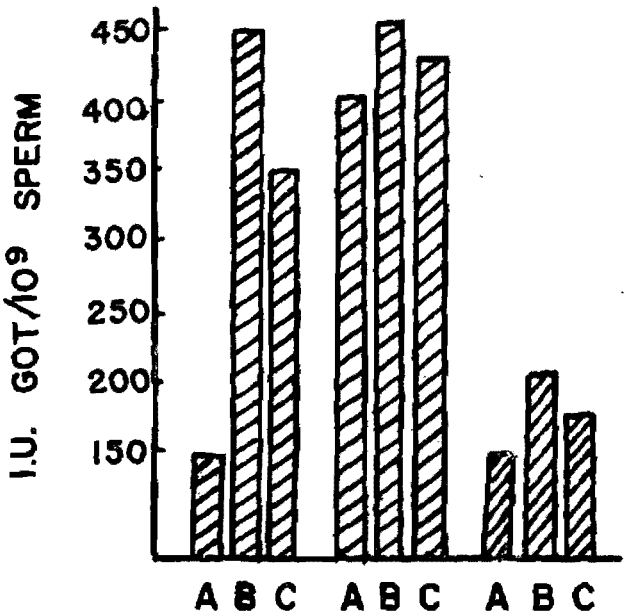

Fig. 11 : An example of the use of enzyme analysis in assessing sperm quality of individual sires showing release of enzyme from cell after: A. Minimal cell damage. B. Maximal cell damage. C. Slow freezing[27].

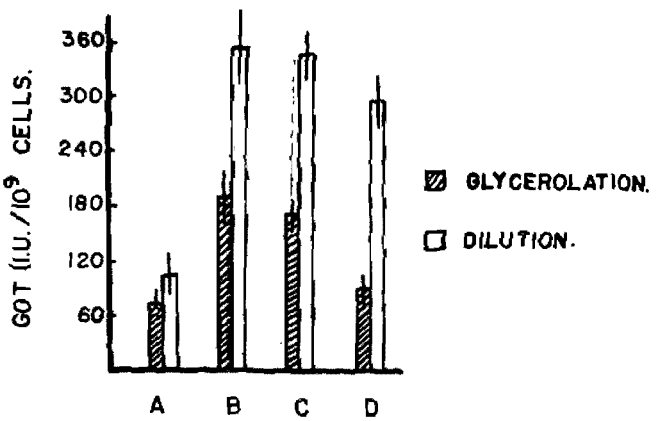

Fig. 12 : The effect of glycerolation of boar spermatozoa in TrisTricine extender and subsequent dilution on GOT release (Means of six ejaculates and their standard errors - A. Control. B. Glycerolated immediately. C. Glycerolated gradually. D. Glycerolated after 2hr. at 5°C)[9].

released from frozen boar spermatozoa as well as changes in acrosomal morphology were indicative of sperm cell damage during freezing[27] (Figs.10 - 13). It appears that motility as the only criterion of viability after freezing is not totally reliable and other more objective tests should be resorted to[13].

It has been shown that thawing of boar semen in seminal plasma resulted in good fertility[15]. It was postulated that some factor in seminal plasma is important in the fertilization process, probably by increasing sperm transport to the site of fertilization. It has been further demonstrated that frozen boar spermatozoa thawed in seminal plasma resulted in far greater numbers of spermatozoa in the oviducts than thawing in TES NaK-glucose buffer[16].

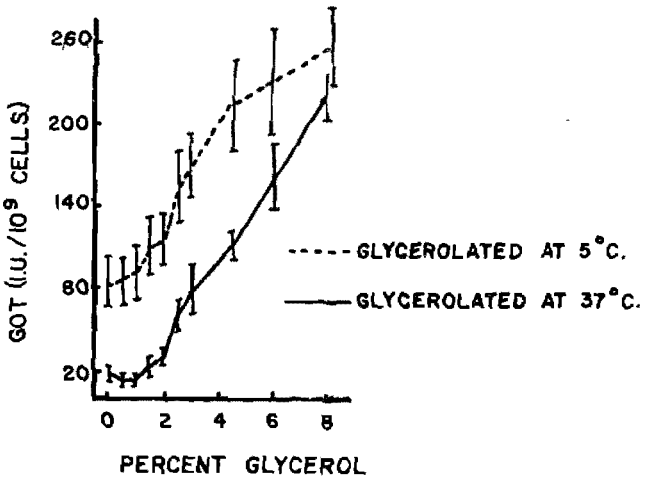

Fig. 13 : The effect of glycerol concentration on GOT release from boar spermatozoa in Tris-Tricine semen extender at 37°C and after cooling (1 - 1½h) to 5°C (Means of four ejaculations and their standard error).

More recently it has been reported that the low fertilization rates obtained with frozen boar semen resulted from insufficient numbers of viable spermatozoa reaching the site of fertilization[68]. They also suggested that further improvement in the freezing procedures will be necessary before frozen boar semen can be used on a commercial basis.

Cognizant of the foregoing it appears that A I in pigs will have to be carried out with ambient temperature diluents where semen is maintained for up to 3 days. This could readily be accomplished by breeding pigs twice during the heat period and doubling the concentration of spermatozoa at each of the subsequent inseminations. Artificial insemination of pigs by the owner on the farm using suitable semen diluents can and should be implemented as the surest way to genetic improvement with sires of superior genetic merit.

RAM AND GOAT SEMEN PRESERVATION

Freezing of ram semen has been the subject of intensified research for a number of years and has been described by several authors[4 40 41 76-79 90 91]. Despite the interest in semen preservation from rams, the commercial application of A I even with fresh semen has not been realized. This is probably due to a number of factors - the economic considerations may not be great enough to warrant the expense of freezing and storage of semen from rams of superior genetic merit. It is also possible that the fertility obtained from frozen ram semen has been discouraging. Gordon[23] reported that storage of semen for even as short a period as one day resulted in a fairly marked decrease in fertility. He suggested that frozen ram semen was not a practical proposition as yet and the most favourable possibility appeared to be that of using high quality semen immediately after collection.

The storage of goat semen used under conditions of A I has been described by a number of authors[22 38 62]. In a general review on freezing of goat semen it has been

suggested that dairy goat semen must be carefully prepared for freezing if 40% of the sperm cells in each sample are expected to survive the freezing procedure and retain their fertilizing capacity[6]. Various steps for the preparation of dairy goat semen for freezing were also suggested,. The freeze preservation of goat buck semen in paillettes using a modified TRIS extender has been recently described[33]. The freezing method resulted in thawing rates of between 60 and 70% motile spermatozoa while the goat insemination trials resulted in a 69% kidding rate.

It is apparent that ram and goat semen can be preserved in the frozen state for a considerable period of time and can be subsequently used in conjunction with oestrous control in an AI programme. However, the results, although sometimes encouraging, are not consistent enough to warrant commercial application on a wide scale. Further research in insemination techniques, timing of insemination, more suitable freezing media and freezing procedures must be carried out.

STALLION SEMEN PRESERVATION

Today in our era of motorization and mechanization it would appear that the horse has been relegated to a insignificant role except in some countries having special conditions which necessitate the use of horses and mules for farm work. However, the horse in more recent times, has gained a new significance because of the increasing popularity of horse-back riding. In the U.S.A. horses and horse related industries are increasing rapidly. The explosion in the equine population has boomed an industry valued at several billion dollars. Horse races attracted over 65 million spectators to U.S.A. racecourses in 1970[63]. Furthermore horses are widely used for recreation and are becoming increasingly important to the well-being of people all over the world. The indications are that this trend will continue to expand to even greater heights in view of the impending energy shortage dilemma. People like to speculate on the use of A I and frozen semen of stallions who have gained prominence on the race course. For instance the cost of a service from the Triple-Crown winner in the U.S.A. is probably in excess of 100 thousand dollars. Despite the obvious monetary advantages accruing from A I and from the use of frozen semen the possibility of using these techniques on thoroughbred horses seems remote.

Freezing of stallion semen has been described by several authors[37 55 56 58 59 71 74]. Satisfactory fertility results have been obtained using frozen stallion semen. Up to 50% fertility has been obtained consistently if the semen was frozen in pellets and more than one insemination performed. Stallion semen is similar in many respects to boar semen both physically and chemically[44]. However, the problem with A I in mares is further compounded because of the long duration of the heat period and the difficulty in predicting ovulation. In light of the fact that timing of ovulation is of vital importance to the successful implementation of A I in horses, it is imperative that more than one insemination is performed during the heat period, especially when frozen semen is used[88]. The possibility of using prostaglandin F2α to induce ovulation shows great promise and may open up many new possibilities for A I in horses.

In Paris, during the 6th International Congress on Animal Reproduction and Artificial insemination the following resolution was adopted by a world wide panel of experts on horse reproduction: That the jockey club or other authorities controlling the registration of thoroughbred horses should be approached in an endeavour to persuade them to accept the registration of progeny resulting from A I under safeguards. These safeguards should ensure adequate veterinary supervision at all stages of the technique, the identification of offspring by blood typing and freedom of the stallion from disease.

It was felt that the advantages of disease control; the saving of costs, and the possible deep-freeze storage of semen for use after the animal's death far outweigh the possible disadvantages.

POULTRY SEMEN PRESERVATION

Throughout the world most of the turkeys are bred using A I — not because of the genetic merit to be gained through A I but primarily because of the size of the males which through genetic selection are no longer able to perform natural mating. Therefore every effort is made to use a suitable diluent for semen extension and the possibility of long term storage of frozen poultry semen has been the subject of intensive investigation[39 43 51 70 89 93].

Turkey and fowl semen can be frozen in a similar manner to semen from other species resulting in satisfactory post freeze motility but the fertility from frozen poultry semen is too low to be considered even for limited commercial application. It has been demonstrated that if the spermatozoa transport to the storage glands was assessed from fresh, frozen and dead turkey spermatozoa, transport was in the order of a 4:1:0 ratio, respectively[51]. It was further demonstrated that if the frozen spermatozoa were placed in the infundibulum, that the resultant fertility was comparable to the control fresh semen group. These findings indicate that even though good motility may be obtained after freezing and thawing the possibility of thawed turkey spermatozoa moving to the site of fertilization is negligible. The fact that spermatozoa have to remain viable within the reproductive tract of the turkey and fowl for such long periods of time after insemination militates greatly against the use of frozen spermatozoa which are known to be less viable than those in fresh semen. The possibility of using frozen poultry semen is further compounded by the problem of a suitable cryoprotective agent for freezing. Glycerol, which is the universal cryoprotective agent for semen freezing has contraceptive activity when added to fowl semen even at a level of 1%[50]. Both glycerol and egg yolk have been reported to be detrimental to fertility in turkeys[70].

Cognizant of these findings it appears that the possibilities for using frozen poultry semen on a commercial scale are remote and further research is warranted in the freezing and thawing of semen in addition to some newer and more favouralbe freezing media.

However, despite the gloomy outlook for frozen poultry semen, a very keen interest has been shown in semen diluents for fresh semen especially that of turkeys. The most commonly used diluents for turkey semen are physiological saline, and Minnesota Turkey Growers Association's diluent. The diluent for turkey semen preservation does not have to be too complex when one bears in mind that the turkeys are inseminated within a short period of time after collection.

The real purpose of the diluent in turkey A I is to extend the semen and facilitate the insemination process which is sometimes cumbersome with neat semen. Furthermore a suitable diluent is a prerequisite for optimal fertility and a diluent with a high energy source would seem most beneficial. TES Na K-fructose-phosphate diluent appears to give the most favourable results under a wide range of conditions[10 28].

With the increasing interest in heavier breeds of turkeys, the use of A I in conjunction with semen dilution and the use of superior toms is inevitable. More interest must be attached to the selection of toms of high fertility and this can be readily accomplished using stress tests in 5°C diluents. The usefulness of frozen semen is recognized and every effort should be made to develop frozen semen which would enable the breeder to select from superior toms during the most favourable season of the year when semen quality is optimum

DOG SEMEN PRESERVATION

The first documented report of fertility from frozen dog semen was of Saeger[75] who reported a pregnancy from semen which had been stored in liquid nitrogen for a period of up to six months. He obtained fertility with a freezing diluent consisting of 11% lactose, 4% glycerol and 20% of egg yolk. Many other diluents have been used[18 34], but Seager's diluent proved to be the most successful.

Recently it was reported that of nine bitches inseminated with frozen semen, only one became pregnant and that was from surgical insemination performed by *laparotomy*[3]. The successful pregnancy resulted from depositing the semen in the corpus uteri immediately after thawing. The bitch conceived and gave rise to three puppies. The semen was frozen in either a Tris extender or a lactose-egg yolk-glycerol extender. The report suggested that conception failure when using frozen semen was primarily connected with the site of deposition of semen rather than with the freezing procedure *per se*. The implication is that the addition of glycerol, the freezing or thawing procedures or some other phenomenon would inhibit a factor necessary for the transport of spermatozoa through the cervix, while the actual fertilizing capacity is retained.

The reports of success with frozen dog semen are sparse but nevertheless encouraging. The proper timing of ovulation would greatly facilitate the use of A I and frozen semen in dogs. In 1971, the journal, "Dog World" reported that the American Kennel Club was not enthusiastic about A I in dogs unless it was practised by a veterinarian[73]. Reasons given for practising A I by a veterinarian were: The possible refusal of some dogs to mate, the danger to the male, as a frightened young bitch might seriously damage a valuable show dog, and the elimination of disease such as canine brucellosis which is transmitted during mating together with other venereal diseases. Storage of dog semen for prolonged periods of time would make possible A I in bitches in all regions of the world. It would greatly widen the gene pool and it would facilitate breeding the "best genes" to the best dogs in the world. "The possibilities are exciting and beyond our imagination" says Saeger[75], who predicts that within 10 years A I and perhaps frozen semen will be used in 80% of our purebred dogs.

HUMAN SEMEN PRESERVATION

No discussion on semen preservation would be complete without reference to the upsurge of human semen banking (sperm banks) which has flourished throughout the world and particularlay in the U.S.A. in the past 2 years. The reason for the sudden upsurge was the possibility of storing semen from men who were contemplating vasectomies as one of the "surest and safest means of birth control". The possibility of having further children after vasectomy from frozen semen helped allay the fears of potential patients and indirectly encouraged birth control by this method. Another reason was the possibility of storing samples from oligospermic males, whereby concentrating and freezing a number of samples, the required number of sperm cells could be inseminated at the appropriate time.

The first report of fertility from frozen human spermatozoa was in 1953 by Bunge and Sherman[11]. From 1953 until 1971 very few reports on frozen human semen were recorded[12 52 61 84].

In 1972, at the first American Seminar on Artificial Insemination in humans, Sherman reported that over 400 births had resulted from frozen human semen stored for varying periods of time[85]. He also reported that human semen which had been frozen for up to 10 years resulted in the birth of a live baby. It was also reported that the amount of DNA remained constant during 6 years of storage. This is of great significance indicating as it does the absence of evidence of induced genetic changes due to freezing.

The intellectual climate for the initiation of a scientific programme directed at a concentrated long term evaluation of human semen banks appears to be developing.

FUTURE HORIZONS FOR CRYOBIOLOGY

The fortutious discovery that semen could be suspended in a frozen state for prolonged periods of time, has marked a turning point in genetics and a new milestone in pushing back the frontiers of knowledge[65]. In 1973, Wilmut and Rowson recorded another milestone in the history of science with the remarkable discovery that an embryo which had been stored in liquid nitrogen for a period of 6 days resulted in the birth of a healthy normal calf[94].

The possibilities that might arise from the storage of embryos are too staggering to centemplate. It would greatly facilitate transport of exotic genetic material between countries. It would also be possible to use frozen embryos from superior parents and incubate them in incubator cows. Frozen embryos could streamline the possibility of each cow giving birth to twins whereby the embryo is transferred into the uterine horn contralateral to the pregnant horn. The fact that spermatozoa and more recently ova may now be stored in liquid nitrogen for very long periods means that the clock can be stopped indefinitely and offspring can result from parents who are long dead. Still another more exciting possibility is the freezing of body cells, say from the buccal cavity with a (2n) chromosome complement and transferring this somatic cell nucleus into an egg which has had its (n) complement nucleus destroyed by irradiation. In this manner the egg is "fooled" into "thinking" that fertilization has taken place and goes on to develop as a normal embryo. The

above argument is purely conjectural but a modicum of success has been achieved with amphibians and carrots. However, it is only a matter of time before scientists will apply this phenomenal and exciting technique to animals, whereby through a process called cloning, an exact replica in every single respect can result from a cell which has been frozen for thousands of years. Cloning in animals has possibilities beyond our imagination and the prospect of breeding thousands of superior animals with the exact same genotype would have far reaching consequences for animal production. The technique of freezing of somatic cells has been perfected and the major limiting factors appear to be concerned with the manupulation of the egg. It therefore behoves scientists throughout the world to pursue vigorously these lines of research as the genetic progress to be derived therefrom is awesome. The possibilities for the freezing of bodies, organs, blood cells, spermatozoa, ova and somatic cells are vast and the field of cryobiology appears to have bright horizons in the years ahead.

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THE CONTROL OF ADULT PARASITIC NEMATODES OF CATTLE WITH MORANTEL TARTRATE*

P.J.S. ANDERSON AND F.S. MARAIS**

SUMMARY

The anthelmintic efficacy of morantel tartrate at 5mg/kg bodymass was investigated in three separate controlled trials comprising 68 calves.

High anthelmintic activity was established against adult *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp. *(C.pectinata and C. punctata), Bunostomum phlebotomum and Oesophagostomum radiatum.*

INTRODUCTION

Morantel tartrate has been shown to be a highly effective anthelmintic in sheep and goats[1 2]. In combination with diethylcarbamazine to control *D. viviparus,* morantel tartrate proved to have high activity against gastriontestinal nematodes of calves in trials conducted in the United Kingdom[3].

It is generally accepted that cattle readily develop a fair resistance to parasitic nematodes after initial infestation. It follows therefore that immature stages of these parasites are usually of greatest importance at first infestation, consequently it was decided that morantel tartrate should be tested against adult nematodes only.

To avoid possible interference between *H. placei* and *O. ostertagi* in the abomasum, separate groups of calves were used for investigating the efficacy of morantel tartrate against these species. Efficacy trials on *B. phlebotomum* and *O. radiatum* were run in both groups to make full use of the experimental animals.

EXPERIMENTAL PROCEDURE

Suitable experimental groups of calves for controlled anthelmintic tests were created by the method described by Reinecke[5]. Susceptible and for all practical purposes, worm-free animals were repeatedly dosed orally with infective larvae of *Haemonchus placei, Ostertagia ostertagi, Oesophagostomum radiatum* and *Cooperia* spp. (*C. pectinata* plus *C. punctata*). Infective larvae of *B. phlebotomum* were administered as a single percutaneous dose.

Treatment with morantel tartrate at 5mg/kg bodymass was administered by stomach tube to ensure that the anthelmintic did not enter the abomasum prior to dilution with the ruminal contents.

At autopsy the gut was divided into three parts: the abomasum, the small intestine and the caecum plus colon, which were handled separately. Each part was opened and the ingesta washed into a bucket. The mucosa of each section was then thoroughly washed into a separate bucket using water under pressure and rubbing by hand ensuring that the whole surface received attention. This last procedure was then repeated twice. When *O. ostertagi* were present this mucosal washing was repeated four times with comprehensive scraping with a metal spatula to remove worms closely associated with the mucosa. Strong Iodine solution (45%) was added to the contents of the various buckets to kill all worms present, and the contents were then passed through sieves with 150 micron apertures. The residues were retained for further examination.

Total worm counts were made from all specimens collected. Recovered worms were retained for identification. For the purpose of these trials only fifth stage worms and adult worms have been recorded in the tables.

Worm recoveries from control and treated calves were subjected to statistical analysis by the method described by Groeneveld & Reinecke[4] as modified by Clark and described by Reinecke[5].

TRIAL I: EFFICACY AGAINST *H. placei, B. phlebotomum* and *O. radiatum*

Materials and Methods

Twenty-three calves of mixed dairy breeds and varying in age from 3 to 5 months were divided at random into two groups and were infested, treated and slaughtered according to the schedule in Table 1.

Table 1: TRIAL I EXPERIMENTAL DESIGN

	No. of infective larvae dosed to each calf		
Day	*H. placei*	*B. phlebotomum*	*O. radiatum*
−44			152
−42			182
−39		4 000	155
−37			170
−35			105
−34			212
−32	306		102
−31			107
−30	359		106
−29			110
−26	353		101
−27	552		210
−25	275		97
−24	326		108
−23	302		102
−22	291		113
−21	302		106
−20	604		
−18	304		
−17	296		
−16	294		
−15	306		
TOTAL	4 870	4 000	2 238
0	Treat 12 calves		
+ 3	Slaughter 4 treated and 3 control calves		
+ 4	Slaughter 8 control calves		
+ 5	Slaughter 8 treated calves		

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Infective larvae of *B. phlebotomum* were applied to the skin of the calves 39 days before the day of treatment ensuring that only fifth stage (young adult) worms would be present at the time of treatment.

Results

Worm recoveries, ranked for statistical analysis from control and treated calves are recorded in Table 2.

Table 2: TRIAL I STATISTICAL COMPARISONS OF ADULT WORM BURDENS

TOTAL NUMBERS OF WORMS RECOVERED					
Haemonchus placei		*Bunostomum phlebotomum*		*Oesophagostomum radiatum*	
Controls	Treated	Controls	Treated	Controls	Treated
589	1	5	0	0	0
694	1	5	0	8	0
725	1	10	0	29	0
737	2	13	0	58	0
760	2	13	0	67	0
771	6	17	0	77	0
793	7	17	0	88	0
846	11	20	0	100	0
1 045	34	22	0	145	0
1 091	68	29	0	168	0
1 114	73	57	0	257	0
	86		0		0
Median of the controls is 771 771(0,25) = 192 No treated calves retained 192 or more worms A grading obtained		Median of the controls is 17 17(0,25) = 4 No treated calves retained 4 or more worms A grading obtained		Median of the controls is 77 77(0,25) = 19 No treated calves retained 19 or more worms A grading obtained	

H. placei

Even worm burdens, varying from 589 to 1 114, with a median recovery of 771 worms, were established in the 11 control calves.

Few *H. placei* were recovered from the 12 treated calves, seven of which retained less than 10 worms. The variation in recovery was from 1 to 86 worms.

On statistical analysis an A efficacy grading was obtained.

B. phlebotomum

The median value of the worm recoveries from the 11 control calves was 17, with a variation from 5 to 57 worms.

No worms were recovered from the 12 treated calves.

On statistical analysis an A efficancy grading was obtained.

O. radiatum

Worm recoveries from the 11 control calves varied from nil to 257 worms. The median was 77 worms.

No worms were recovered from the 12 treated calves.

On statistical analysis an A efficacy grading was obtained.

TRIAL II: EFFICACY AGAINST *O. ostertagi*, *B. phlebotomum* and *O. radiatum* and *Cooperia* spp.

Materials and Methods

Twenty-two calves of mixed dairy breeds and varying in age from 3 to 5 months were divided at random into two groups and were infested, treated and slaughtered according to the schedule in Table 3.

Infective larvae of *B. phlebotumum* were applied to the skins of the calves 59 days before the day of treatment to ensure that the worms were adult at the time of treatment.

Results

Worm recoveries, ranked for statistical analysis, from control and treated calves are recorded in Table 4.

Although not planned, fair numbers of *Cooperia* spp. were recovered from these calves, and are ranked in Table 5 for statistical analysis.

O. ostertagi

Fairly even worm burdens were established in the 10 control calves, varying from 199 to 486 worms, with a median recovery of 356 worms.

Recoveries of this species from the 12 treated calves varied from 38 to 366 worms. On statistical analysis a B efficacy grading was obtained.

B. phlebotomum

The median of the worm recoveries from the 10 control calves were 18, varying from 8 to 39 worms.

Three worms were recovered from one treated calf.

Table 3: TRIAL II EXPERIMENTAL DESIGN

Day	No. of infective larvae administered to each calf		
	O. ostertagi	*B. phlebotomum*	*O. radiatum*
−59		3 336	
−49			600
−42			476
−36	600		
−35			98
−34			66
−32	400		100
−31			104
−30			108
−29			107
−28	416		92
−27			134
−25			99
−24			106
−23	214		95
−22	294		100
−21	294		98
−20	667		
−18	290		
−17	317		
−16	304		
−15	302		
−14	294		
−13	294		
−11	296		
TOTAL	4 982	3 336	2 433

0	Treat 12 calves
+ 3	Slaughter 8 control calves
+ 4	Slaughter 2 control and 6 treated calves
+ 5	Slaughter 6 treated calves

In the remaining 11 treated calves no *B. phlebotomum* were retained. On statistical analysis an A efficacy grading was obtained.

O. radiatum

Worm recoveries from the 10 control calves varied from 1 to 59, with a median recovery of 36 worms.

No worms were recovered from the 12 treated calves.

On statistical analysis an A efficacy grading was obtained.

Cooperia ssp.

Table 5: TRIAL II STATISTICAL COMPARISON OF ADULT *Cooperia* spp. WORM BURDENS

No. of adult *Cooperia* spp. revovered.	
CONTROLS	TREATED
4	0
7	0
82	0
111	0
150	0
181	0
196	0
338	0
352	0
404	1
	1
	2

Median of the controls is 165
165 (0,25) = 41

None of the treated calves retained 41 or more worms

A grading obtained

Table 4: TRIAL II STATISTICAL COMPARISONS OF ADULT WORM BURDENS

TOTAL NUMBERS OF WORMS RECOVERED					
Ostertagia ostertagi		*Bunostomum phlebotomum*		*Oesophagostomum radiatum*	
Controls	Treated	Controls	Treated	Controls	Treated
199	38	8	0	1	0
231	52	11	0	2	0
313	61	11	0	13	0
321	73	11	0	16	0
348	81	13	0	36	0
364	85	24	0	37	0
364	86	28	0	41	0
466	109	28	0	46	0
471	114	28	0	50	0
486	123	39	0	59	0
	180		0		0
	366		3		0
Median of the controls is 356 356(0,25) = 89 356(0,4) = 142 2/12 treated calves retained 142 or more worms B grading obtained		Median of the controls is 18 18(0,25) = 4 No treated calves retained 4 or more worms A grading obtained		Median of the controls is 36 36(0,25) = 9 No treated calves retained 9 or more worms A grading obtained	

Worm recoveries from the 10 control calves varied from 4 to 404, with a median recovery of 165 worms.

A treated calf harboured two worms, while two calves each retained one worm. The remaining nine calves in the treated group did not retain any of these worms after treatment.

On statistical analysis an A efficacy rating was obtained.

Discussion

The experimental calves in trails I and II were raised on concrete. The ration consisted of calf growth pellets and hay. *Cooperia* spp. may have been introduced in the dried hay but it seems more probable that the odd mouthfull of Kikuyu grass picked up on the way to and from the cattle crush was the mode of infestation. This is in keeping with the fact that the calves in Trial II had further to go to the crush than those in Trial I and they have developed a more substantial burden of *Cooperia* spp. than did those in Trial I. In Trial I a maximum of 14 *Cooperia* spp. were recovered from a calf with the majority of burdens below 10 worms. These worms were not recovered from the treated calves in Trial I.

TRIAL III: EFFICACY AGAINST *Cooperia* spp.

Material and Methods

Twenty-three calves of mixed breeds and varying in age from 6 to 10 months were divided at random into two groups and were infested, treated and slaughtered according to the schedule in Table 6.

Table 6: TRIAL III EXPERIMENTAL DESIGN

No. of infective larvae dosed to each calf	
DAY	*Cooperia* spp.*
−20	509
−19	500
−18	500
−17	1 016
−14	1 061
−12	1 008
−11	506
−10	506
− 9	508
TOTAL	6 114
0	Treat 10 calves, slaughter Day 0 control
+ 21	Slaughter 4 control calves
+ 22	Slaughter 4 control calves
+ 23	Slaughter 4 control calves
+ 28	Slaughter 4 treated calves
+ 29	Slaughter 4 treated calves
+ 30	Slaughter 2 treated calves

* *Cooperia pectinata* and *Cooperia punctata*

The control and treated groups of calves were slaughtered approximately three weeks after the day of treatment to facilitate recovery of fully developed worms.

Results

Total worm recoveries ranked for statistical analysis are recorded in Table 7.

Table 7: TRIAL III STATISTICAL COMPARISON OF ADULT *Cooperia* spp. WORM BURDENS

No. of adult *Cooperia* spp recovered	
CONTROLS	TREATED
31	0
263	2
279	10
336	15
1 510	21
1 831	32
2 488	35
2 541	54
2 732	104
3 593	105
4 014	
4 060	

Median of the control is 2 160
2 160 (0,25) = 540

No treated calves retained 540 or more worms

A grading obtained

Cooperia spp.

The median number of worms recovered from the 12 control calves was 2 160, varying from 31 to 4 060 worms.

Not one of the 10 treated calves harboured more than 105 worms.

On statistical analysis an A efficacy rating was obtained.

CONCLUSIONS

In the light of the above findings the anthelmintic efficacy of morantel tartrate at 5mg/kg bodymass can be clasified as follows in terms of the requirements of the Registering Officer (Act 36 of 1947, Republic of South Africa).

WORM SPECIES	ADULT WORMS
Haemonchus placei	A
Ostertagia ostertagi	B
Cooperia spp. *(C. pectinata* and *C. punctata)*	A
Bunostomum phlebotomum	A
Oesophagostomum radiatum	A

KEY	
CLASS	DEFINITION
A	More than 80% effective in more than 80% of the treated herd.
B	More than 60% effective in more than 60% of the treated herd.
C	More than 50% effective in more than 50% of the treated herd.
X	Ineffective.

By comparing the means of the adult worm recoveries from control and treated groups of calves in each trial the anthelmintic efficacy of morantel tartrate, in terms of percentage kill, was found to be as follows:

WORM SPECIES	TRIAL I	TRIAL II	TRAIL III
H. placei	97%		
O. ostertagi		68%	
Cooperia spp.		99%	98%
B. phlebotomum	100%	99%	
O. radiatum	100%	100%	

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THE EFFECT OF DIFFERENT DIETARY LEVELS OF Ca AND P ON THE PLASMA Ca, INORGANIC P AND Mg AS WELL AS THE ASH, Ca, P AND Mg CONTENT OF CERVICAL VERTEBRAE AND TOTAL BODY OF SHEEP

N. McDONALD AND P.C. BELONJE*

SUMMARY

Six 14 month old rams were divided equally into three groups and received either a high Ca (1,102% Ca : 0,192% P : 0,128% Mg), control (0,322% Ca : 0,311= P : 0,128% Mg) or high P (0,127% Ca : 1,043% P : 0,130% Mg) diet in pelleted form for 150 days.

2. Dry mass and ash % of the third cervical vertebra decreased as Ca intake decreased while fat % increased. There was a highly significant negative correlation (= -0,992) between bone ash% and bone fat %. Bone P and Mg showed no particular trends.

3. Total body Ca and P as well as % body Ca and P all decreased with a decrease in dietary Ca and increase in dietary P intake. Body Mg showed no particular trend.

4. The results suggest that while plasma values may be useful in assessing the P intake of sheep on natural pastures, bone and total body P may not be.

INTRODUCTION

The natural pastures of South Africa have been shown to be deficient in P[3]. As the sheep is a selective grazer, however, handcut specimens are not representative of the quality of the material which is taken in by sheep. So, for instance, Engels (1972)[5] has found that the plant material gathered from oesophageal fistulated sheep contained, on average, 125,7 percent more crude protein, 37,6 percent less crude fibre and 43,3 percent more digestible organic material than specimens cut by hand from the same pasture. In order to assess the amount of P in natural pastures as selected by sheep it is therefore necessary either to analyse material gathered from oesophageal fistulated sheep or to analyse the sheep itself. Unfortunately contamination by salivary P makes the former impractical at present and consequently animal analysis has to be done. Moreover, as there is an interrelationship between P, Ca, and Mg the latter two must be included in any investigation on P.

The early work on P in sheep was confined mainly to body measurements and blood analyses[1 4 7 10]. The blood values have been shown to give a reasonable indication of the dietary intake of Ca and P but give no indication of the amounts in the body. For this reason then, the present trial was conducted with sheep on pelleted rations which contained different levels of Ca and P but approximately the same levels of Mg, and analyses were done on blood, bone and the total carcass.

Recent work[2] has shown that the bones of the sheep do not resorb at the same rate and that the susceptibility to resorption decreases in the following order: vertebrae; pelvis; skull; sacrum; mandible; ribs; proximal end of tibia; scapula; sternum; proximal and distal ends of the humerus, radius and femur; distal end of tibia; shafts of humerus, radius femur and tibia; metatarsals and metacarpals. It was therefore decided to use the vertebrae as an indication of bone mineralization and the third cervical vertebra was chosen as it is easy to identify and always present in the slaughtered animal.

Furthermore, as we wished to analyse for bone fat and also minimize possible loss of soluble bone minerals, the usual technique of boiling in water and defatting was not used. The bones were freeze dried and then milled while frozen and the results expressed on a dry mass basis.

In addition, in order to assess the effects of the different diets on the total body composition of Ca, P and Mg, the total carcass, not including the cervical vertebra and gut contents, but including the blood, was milled fine and analysed.

MATERIALS AND METHODS

Animals

Six 14-month old rams were used. They were housed in a shed in individual pens with slatted floors for the four month experimental period, at the end of which they weighed an average of 57,4± 5,24 kg fasted body mass.

Rations

Morrisson's (1958) tables[8] were used to prepare a basic ration with 70% total digestible nutrients and 14,9% digestible crude protein (see Table 1). The trace elements were added in the form of their sulphate salts.

Table 1: THE COMPOSITION OF THE BASIC RATION

Component	Amount (%)
Maizemeal (or Samp)	55
Oat hay	30
Blood meal	9
Peanut oil cake meal	5
Salt	1
Copper (as Cu)	5 ppm
Zinc (as Zn)	50 ppm
Manganese (as Mn)	50 ppm

* Dept. Animal Physiology, University of Stellenbosch, Stellenbosch.

The following three experimental rations were then formulated using $CaCO_3$ and NaH_2PO_4 to modify the Ca and P contents of the basic ration (see Table 2). In the high Ca low P diet, samp was used in place of maize meal to lower the P content and widen the Ca: P ratio even further. All three rations were fed in the pelleted form.

Table 2: THE COMPOSITION OF THE FINAL THREE RATIONS

Component	High Ca ration	Control ration	High P ration
Basic ration	1 000	1 000	1 000
$Ca\ Co_3$	30	6,4	–
$Na\ H_2\ Po_4$	–	5,1	40

Representative samples of these rations were taken and analysed by spectrochemical means to determine the Ca, P and Mg levels.

Experimental procedure

During the entire experimental period the sheep received sufficient food to satisfy their maintenance requirements[8]. For 28 days all the animals were fed the control ration in order to adapt. Thereafter two animals were placed on each of the three different diets and remained on them for 150 days. Water was always available except for two hours before blood was taken.

At the end of the experimental period the animals were shorn and on the 150th day they were slaughtered and specimens were collected for analyses.

Specimens and analytical methods

(a) Blood analyses: On the 108th, 122nd and 136th day of the experimental period free flowing jugular blood was collected in disposable plastic syringes, transferred to heparinized tubes and centrifuged for plasma. Plasma inorganic phosphate analyses[12] commenced within half an hour of blood withdrawal. The remaining plasma was stored at -20°C in airtight glass bottles until analysed for total plasma proteins by means of a Technicon auto-analyser[12]; plasma calcium and magnesium were determined on a Perlkin Elmer atomic absorption spectrophotometer after diluting the plasma 1:20 with 0,1% La and read against Hopkin and Williams standards suitably diluted with 0,1% La.

(b) Bone analyses: The third cervical vertebra was used for these determinations. After the animals had been killed, this vertebra was dissected free and then carefully cleaned of all extraneous tissue. The vertebrae from the six animals were then dried in a freeze drier for 72 hours at a pressure of 0,7 torr and a maximum temperature of 60°C. The dried bones were then weighed, broken into small pieces and mixed with about five times their own mass of dry ice. After about five minutes the frozen pieces were milled together with more dry ice in a Christie and Norris laboratory mill. This technique of freezing the bones prevents the loss of tissue, particularly fat, during milling. Each individual milled bone sample was stored at – 20°C in plastic bags until analysed.

(i) Bone fat analysis: Two specimens of about 2g, from each sample were accurately weighed. Fat was determined on the one specimen by means of a Goldfish apparatus, while the other specimen was air dried in an oven at 103°C for 12 hours to determine the moisture content.

This allowed the fat percentage to be expressed on an oven dry basis.

(ii) Bone ash analysis: Two specimens, of about 2g, from each sample were accurately weighed. Moisture content was determined on the one as above, while the other specimen was first charred over an open flame and then ashed at 550°C for five hours. After cooling in a dessicator over silica gel the ash percentage on an oven dry basis could be determined.

This same ash was dissolved in 30 ml concentrated HCl over a boiling waterbath and transferred quantitatively into a 100 ml volumentric flask and made up to the mark with deionized distilled water. This stock solution was analysed for P, Ca and Mg.

(iii) Bone phosphorus analysis: A 20 times dilution of the stock solution was made during the determination of P by means of the phospho-vanado-molybdate method of Hanson (1951)[6]. Readings were made on an Metrohm E 1009 spectrocolorimeter.

(iv) Bone calcium analysis: A 1:200 dilution of the stock solution was made with 0,1% La and Ca and Mg were determined on a Model 440 Beckman atomic absorption spectrophotometer against Hopkin & Williams standards suitably diluted with 0,1% La.

(c) Carcass analyses: The animals were weighed and after they were killed by slitting their throats, the blood was collected in clean plastic vessels. The gastro-intestinal tract was removed, weighed, washed clean and reweighed to determine the weight of the intestinal contents. The whole carcass including the blood, but excluding the cervical vertebra was then sent through a Wolking mill. The mixing and milling process was repeated four times to ensure homogeneity of the specimens.

A specimen of about 600g per animal was taken, weighed accurately and freeze dried and milled in dry ice as described for bone. Fat, ash, P, Ca and Mg determinations were done as for bone except that only 15 ml concentrated HCl was used to dissolve the ash and the stock solution was made up to only 50 ml.

(d) Statistical analyses: Data were subject to statistical analyses as outlined by Snedecor (1956)[11].

RESULTS

Ration analyses

The spectrochemical analyses for Ca, P and Mg and the actual intake of these minerals per sheep per day appear in Tables 3 and 4 respectively.

Table 3: THE Ca, P AND Mg CONTENT OF THE THREE RATIONS

Ration	Ca %	P %	Mg %	Ca:P ratio
High Ca	1,102	0,192	0,086	5,74 : 1
Control	0,322	0,311	0,128	1,04 : 1
High P	0,127	1,043	0,130	0,12 : 1

Table 4: THE INTAKE OF Ca, P AND Mg PER SHEEP PER DAY

Ration	Ca (g)	P (g)	Mg (g)
High Ca	9,95	1,73	0,8
Control	2,90	2,80	1,2
High P	1,15	9,44	1,2

Plasma analyses

The results of the blood analyses appear in Table 5.

Table 5: THE PLASMA Ca, INORGANIC P, Mg AND TOTAL PLASMA PROTEIN (T.P.P.) VALUES DURING THE EXPERIMENTAL PERIOD

Plasma Constituent	Ration	Sheep No.	Days during experimental period		
			108	122	136
Ca (mg%)	High Ca	1	10,0	11,2	12,2
		2	10,8	11,6	11,0
	Control	3	9,4	9,9	10,8
		4	9,9	10,5	10,2
	High P	5	8,5	10,4	8,0
		6	7,7	7,5	6,8
Inorganic P (mg%)	High Ca	1	3,7	5,6	4,1
		2	2,9	2,8	3,1
	Control	3	6,7	6,9	6,5
		4	5,5	4,1	5,1
	High P	5	6,2	8,9	7,2
		6	6,5	6,8	7,6
Mg (mg%)	High Ca	1	2,1	2,2	2,0
		2	2,1	1,9	2,5
	Control	3	2,5	2,6	3,3
		4	2,3	2,3	3,3
	High P	5	2,7	2,9	3,0
		6	2,0	2,0	2,0
T.P.P. (g%)	High Ca	1	6,98	7,45	7,66
		2	8,05	7,79	8,35
	Control	3	6,98	7,62	7,48
		4	6,62	7,56	7,17
	High P	5	7,11	8,83	6,83
		6	7,15	7,59	8,67

Bone analyses

The results of the bone analyses appear in Table 6.

Carcass analyses

The results of the carcass analyses appear in Table 7.

DISCUSSION

Rations

The spectrochemical analyses of the three rations confirmed that the rations were in fact high Ca : low P; control and high P: low Ca, with Ca : P ratios extending from 5,74:1 1,04:1 to 0,12:1 (see Table 3).

The daily intake (see Table 4) of 2,9g Ca and 2,8g P by the sheep on the control ration approximates closely the N.R.C.[9] recommendations for lambs of about 50 kg live body mass, with the other two rations varying widely from these figures.

Plasma values (see Table 5)

Although there are too few values for statistical analyses there are definite trends. As the dietary intake of Ca decreases so the plasma Ca values decrease and as the dietary P increases the plasma inorganic P also shows an increase. However, neither the plasma Mg nor the T.P.P. values show any particular trends.

Bone analyses (see Table 6)

There was a tendency for the bones of the animals to be lighter as the calcium content of the diets decreased. This is probably due, in part at least, to the decreasing ash content of the bones.

An interesting finding is that there was a highly significant negative correlation between the ash percentage and fat percentage in the bones (correlation coëfficent = -0,992). It is reasonable to assume that in these bones fat replaces the mineral elements during demineralization and *vice versa* during mineralization (see Fig. 1).

There was a significant positive correlation between ash percentage and Ca percentage (correlation coeffiecient = 0,864) with the latter also decreasing with decreasing Ca intakes. However the P percentage shows no trend and was not correlated to either ash percentage or Ca percentage. In fact the P values vary only slightly between all the sheep and appear not to have been affected either by changes in dietary Ca or

Table 6: THE DRY WEIGHT, PERCENTAGE FAT, ASH, Ca, P AND Mg AND THE Ca, P AND Mg AND THE Ca : P RATIO OF THE THIRD CERVICAL VERTEBRAE

Ration	Sheep No.	Dry bone weight (g)	Fat(%)	Ash(%)	Ca(%)	P(%)	Mg(%)	Ca : P ratio
High Ca	1	40,5709	9,67	54,27	21,75	10,10	0,30	2,15 : 1
	2	48,7983	9,89	53,79	21,89	10,26	0,28	2,13 : 1
Control	3	45,7093	10,95	53,46	20,73	10,41	0,38	1,99 : 1
	4	41,4840	12,94	51,23	20,43	10,11	0,36	2,02 : 1
High P	5	39,6491	16,53	49,13	19,79	10,31	0,38	1,92 : 1
	6	33,9114	15,49	49,54	18,08	10,13	0,36	1,78 : 1

Table 7: THE DRY, INGESTA-FREE BODY WEIGHT, ASH %, TOTAL BODY Ca, P AND Mg AND PERCENTAGE Ca, P AND Mg IN THE DRY BODY

Ration	Sheep No.	Dry body weight (kg)	Ash %	Total Ca(g)	Total P(g)	Total Mg(g)	Ca %	P %	Mg %
High Ca	1	20,63	9,58	755,3	460,0	14,4	3,66	2,23	0,07
	2	20,59	9,58	792,7	378,0	18,5	3,85	1,84	0,09
Control	3	21,45	9,52	699,2	360,4	17,2	3,26	1,68	0,08
	4	20,01	10,34	644,3	352,2	26,0	3,22	1,76	0,13
High P	5	17,87	9,16	475,3	302,2	17,9	2,66	1,69	0,10
	6	20,39	8,61	430,0	218,2	12,2	2,11	1,07	0,06

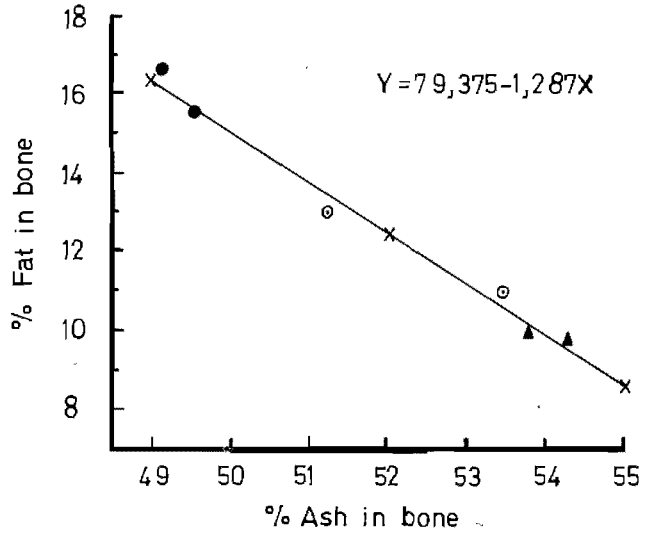

Fig. 1: Showing the HIGHLY significant negative correlation between bone ash percentage and bone fat percentage.
◗ = Sheep on high Ca diet
○ = Sheep on control diet
● = Sheep on high P diet.

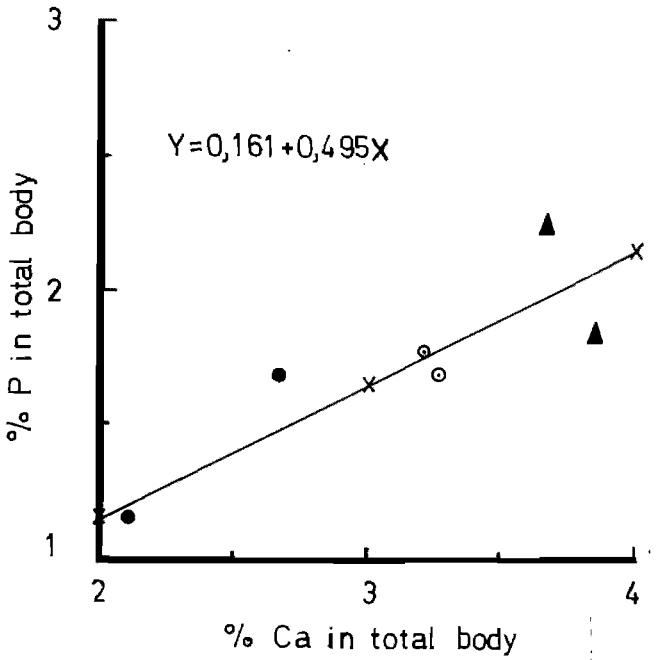

Fig. 2: Showing the significant positive correlation between total body Ca percentage and total body P percentage
◗ = Sheep on high Ca diet
○ = Sheep on control diet
● = Sheep on high P diet.

P. As the Ca values did show an effect, this explains the decreasing Ca : P ratios which were found.

Once again the Mg percentage did not show any particular trend although the sheep receiving the high Ca diet which contained the least Mg had values slightly lower than the rest.

Carcass analyses

There were no large differences between the dry, ingesta-free, body masses of the animals, and there was only a slight downward trend in ash percentage as the Ca intake decreased. Furthermore no particular trend can be discerned in the Mg values. There were marked differences however, between the groups in the total body Ca and percentage body Ca decreased. Furthermore, as the dietary Ca decreased and the dietary P increased there was also a decrease in total body P and percentage body P. This is in contrast to the plasma where the Ca and inorganic P values reflected the dietary pattern and in the bones where P did not follow the Ca pattern.

There was a significant positive correlation (correlation coefficient = 0,854) between the decreases in total body Ca percentage and total body P percentage as the dietary Ca intake decreased and the dietary P increased. This correlation is presented graphically in Fig. 2. The deviations of the actual points from the regression line were tested for significance and it was found that they fit the regression line significantly at the 5% level (t=3,257). From these findings it is clear that in contrast to Ca one is not able to predict the dietary P intake from the P content of the total body as this appears to depend to a large degree on the Ca intake. Moreover, the bone analyses in this particular study also gave an indication of Ca intake but again did not indicate the P intake. On the other hand the blood values were a reflection of the dietary intakes of both Ca and P.

As the sheep which received a diet with a Ca : P ratio of 5,74 : 1, containing only 0,192% P, had more total P than those receiving a diet with a Ca : P ratio of 0,12 : 1, containing 1,043% P and as the body P was significantly correlated to body Ca, which in turn was determined by Ca but not P intake, it is reasonable to assume that Ca plays the more dominant role in the utilization of these two minerals. It may however mean that on a high Ca diet there is far more bone mineralization and that, although there is more P in the total body, this is trapped in the skeleton and the soft tissue may in fact have a low P content. The effect of these types of diets on the P content of soft tissues is most important and is being investigated at present.

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PENTASTOMIASIS (**Armillifer** AND **Linguatula** Sp.) INFESTATIONS OF WILD ANIMALS IN THE KRUGER NATIONAL PARK

E. YOUNG*

SUMMARY

Armillifer armillatus, Linguatula serrata and *L. nuttalli* have each been isolated from nine different mamalian species in the Kruger National Park: lion, *Panthera Leo;* Leopard, *P. pardus;* buffalo, *Syncerus caffer:* blue wildebeest, *Connechaetes taurinus;* giraffe, *Girraffa camelopardalis;* kudu, *Tragelophus strepsiceros;* waterbuck, *Kobus ellipsyprymnus;* tsessebe, *Damaliscus lunatus* and impala, *Aepyceros melampus.* Successful transmission of *L. serrata* from the lion to a domestic ox and impala is recorded for the first time. Pentastomiasis is also a desease of man and these findings are possibly of epidemiological significance.

DISCUSSION

Human pentastomiasis has been recognised for over 100 years in many parts of the world and results from infestation by the Porocephalidae (e.g. *Armillifer armillatus*), a disease known as porocephaliasis, and the Linguatulidae (e.g. *Linguatula serrata*), a condition often called linguatuliasis[4 6].

Armillifer is the best represented genus in the Ethiopian region and *A. armillatus* is the species most frequently affecting humans in Africa[14]. Infestations with *Armillifer* spp. are common in regions in which snakes are ingested as food or medicine[4].

Porter[7] found nymphs of *A. armillatus* in the liver and intestine of a native South African (Shangaan) and Cannon[3] recorded nymphs from a native woman in Nigeria. Goldsmid and Melmed[4] also reported a case of human porocephaliasis in Rhodesia.

A. armillatus is found in its adult form in the lungs of snakes, such as the African python, *Python sebae,* puff adder, *Bitis arietans,* and Gaboon viper, *Bitis gabonica.* The most common host for the adult parasites is the python. The immature forms[5] occur in mammals, i.e. species of the following orders: Primates, Insectivora, Carnivora, Tubulidentata, Artiodactyla and Rodentia[14]. In the Kruger National Park *A. armillatus* was found in a leopard, indicating that this carnivore may occasionally prey on snakes. The occurrence of this parasite in the Eastern Transvaal Lowveld emphasizes the possibility of further cases of human porocephaliasis.

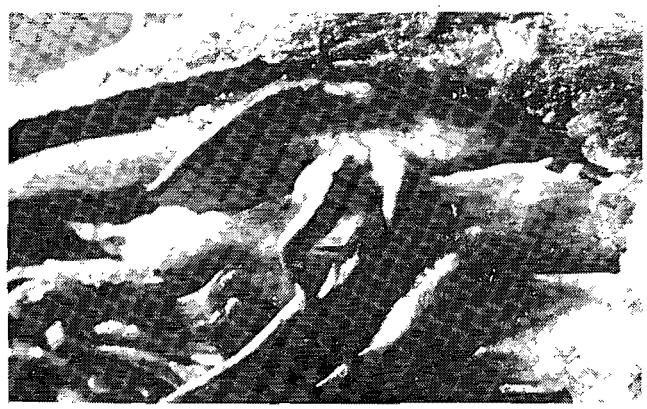
Adult Linguatulidae are usually found in the nasal and respiratory passages of carnivores. Their eggs are passed out with the nasal secretions or in the faeces to infest herbivorous intermediate hosts. The final host aquires the parasite by eating the infested intermediate host. Man can become infested with both the adult and immature forms of *L. serrata*[14].

Adult *L. serrata* has been recorded in South Africa from the domestic dog[14] and from lion in the Kruger National Park[11]. *L. nuttalli,* infesting the pharynx of lions in East Africa[14], has now also been found in lion of the Kruger National Park, and three species of pentastomes are thus known to occur in this wildlife sanctuary.

L. multiannulata has been recorded in spotted hyaena, *Grocuta crocuta,* from East Africa[8]. Examination of at least 20 hyaenas in the Kruger Park failed to reveal the parasite. In this park the lion seems to be the only final host of this genus, harbouring *L. serrata* and *L. nuttalli* only.

In several parts of the Kruger Park most or all of the older lions are infested. The parasites attach themselves high up in the nasal passage and sometimes in the naso-pharynx of this species and produce a severe irritation which causes intermittent sneezing and coughing. A mucous discharge, often blood-stained, can occasionally be seen to exude from the nostrils and affected lions may be seen to rub their noses with their forefeet.

Adult parasites may be responsible for necrotic rhinitis in affected lions. Microscopic examination by Dr. P.A. Basson *(pers. comm.)* revealed loss of epithelial cells, vasculitis and mild round cell infiltration, as well as an increase in collagenous tissue in the propria of the turbinates. In some cases a brownish pigmented fungus, morphologically resembling *Aspergillus,* has been observed to establish itself on the eroded surfaces caused by *Linguatula.* It sometimes invades the tissue and blood vessels to aggravate the primary pentastomal lesions.



Pentastimiasis – K.N. Park – E. Young

Fig. 1: Tongue shaped adult *L. serrata* attached to turbinates of lion.

The nymphs of *L. serrata* have been recorded in cattle in South Africa and Swaziland[14 15 16]. They may also occur in almost all other domesticated species, including different ungulates, dogs, cats, rabbits, rats and guinea pigs, as infestation of these hosts has been recorded in other parts of the world[14].

In the Kruger National Park various wild herbivorous species are infested with the immature forms

* Division of Veterinary Services, Kruger National Park, P.O. Skukuza, 1350

of *Linguatula*. These include buffalo[1 2 11] blue wildebeest[1 11 13], kudu[1], giraffe, waterbuck, tsessebe and impala.

The incidence of infestation is very high in buffalo (60-70%)[11] and blue wildebeest (70-80%)[13], suggesting extremely severe infestation of pastures and drinking places by lions and, perhaps some other unknown final hosts. In contrast to the frequent infestation of adult buffalo, calves are rarely infested[1 11].

In impala the infestation rate is less than 1%[12]. The impala represents by far the most abundant species of antelope in the Kruger Park and does not seem to present a very suitable natural intermediate host for these parasites. The same applies to elephant, *Loxodonta africana*, and Burchell's zebra, *Equus burchelli antiquorum;* of each many hundreds have been examined with negative results.

The pentastome nymphs in buffalo were identified as immature *L. serrata*. As in most intermediate host species the small tongue shaped parasites are found mainly in the cardiovascular system, liver and lymph nodes. They are usually most conspicious in the hepatic veins. In the liver they may be found in the absence of any microscopic pathological lesions. In other cases, liver lesions associated with *Linguatula* infestations include focal necrosis of the liver parenchyme, encystation of the parasites, ulceration of the liver capsule and, in many cases, lesions of chronic fibrous peritonitis on the liver surface resulting in villi-like projections[11].

Only 10% of the affected buffalo revealed microscopic lesions. These were either cystic or granulomatous and contained either live or dead parasites or their shed cuticles. Hepatic cysts containing pentastomes were the most common microscopic lesions observed[2].

The pathogenicity and life cycle of *L. serrata* has been studied by our laboratories: this parasite can be transmitted from the lion to impala and the domestic ox by oral administration of suspensions of disintegrated adult parasites obtained from free living lions. In spite of the apparent resistance of impala to natural infestation, the artificially induced infestation of one impala resulted in a dramatic reaction (emaciation and mortality) and the subsequent recovery of a few hundred immature parasites from its liver.

In contrast, buffalo and blue wildebeest, commonly infested in their free-living state, and two sheep failed to react to the administration of lion parasites; all animals were slaughtered from 3 to 7 months after exposure. These negative results should be confirmed but it seems there may be some unknown factors which prevent transmission of the parasites from the final to the intermediate hosts.

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RESISTANCE OF THE SHEEP BLOWFLY, *LUCILIA CUPRINA* TO INSECTICIDES IN THE REPUBLIC OF SOUTH AFRICA

G.G. BLACKMAN* AND J.A.F. BAKKER**

SUMMARY

Laboratory tests on larvae of nine strains of the sheep blowfly, *Lucilia cuprina*, and one of the hairy blowfly, *Chrysomia albiceps*, taken from the field are reported. Results indicate resistance to arsenic in one strain and to diazinon in the remainder and in *Chrysomia albiceps*. Field evidence corroborates the presence of resistance in the blowfly maggots to current flystrike insecticides with reduced protection resulting in more frequent treatment.

INTRODUCTION

Resistance of the sheep blowfly *Lucilia cuprina* to insecticides has been well documented in Australia[6 7 8]. Dieldrin resistance has also been found in *Lucilia sericata* in New Zealand[4] and Eire[10]. A short review of resistance in blowflies to insecticides by Howell[5] mentioned *Lucilia cuprina* resistance in South Africa to a number of insecticides with factors of resistance ranging from two to one hundred times but no details were given. This paper describes the findings of laboratory tests on *Lucilia cuprina* from the Republic of South Africa.

The pattern of insecticide use for sheep treatment in South Africa has generally followed that of Australia and Great Britain. Arsenic was in common use in South Africa at the turn of the century, with phenolic-type treatments taking a minority position. Dipping generally took place once a year soon after shearing and was directed primarily against keds, lice and scab; blowfly control was thus incidental. An attack of blowfly larvae subsequent to dipping was clipped clean and treated with either arsenic or a phenolic solution to kill off any remaining larvae.

Arsenical treatments were in use up to the time of World War II when first DDT and then BHC were introduced and used extensively. Both chemicals enjoyed widespread usage until about 1953 when the highly effective organo-chlorine insecticide dieldrin was launched, first experimentally, and then on a large scale in 1954. First reports of resistance to dieldrin were received in 1956 and were confirmed in 1957[1].

At this time the first organo-phosphorus (o.p.) insecticides, diazinon, dioxathion and coumaphos, were commercially introduced. These three chemicals were highly favoured until the mid 1960's when chlorfenvinphos, dichlofenthion, and fenthion-ethyl were marketed. Since this time the products of choice have been diazinon, chlorfenvinphos, dichlofenthion and fenthion-ethyl.

The carbamate insecticides have not been used commercially for the treatment of fly strike in South Africa. However, if the development of resistance to insecticides found in Australia also occurs in South Africa, it is likely that cross-resistance to carbamates is already present or would shortly appear.

* Wellcome Research Laboratories, Berkhamsted Hill, Berkhamsted, Hertfordshire, England.

** Kwanyanga Research Station, Coopers (South Africa) (Pty) Limited, Greenfields, East London, R.S.A.

MATERIALS AND METHOD

A number of strains of the sheep blowfly, *Lucilia cuprina* and one of *Chrysomia albiceps*, the hairy blowfly, were submitted *via* the offices of Cooper (S.A.) (Pty) Limited in East London to the Wellcome Research Laboratories, Berkhamsted, England, for resistance assesment. Larvae were taken from infested sheep or baited traps on individual properties and reared in the laboratory in East London before being despatched as pupae by air to England. Flies were bred in the laboratory until the colony was sufficiently large to provide larvae for testing.

Larvae were chosen to assess resistance, because of their greater insecticidal resistance in comparison with the adult[3] to provide a more sensitive test.

The method of assessment used is a modification of that described by du Toit and Fiedler[2] and later by Shaw and Blackman[11] using an automatic diluting apparatus. Since the publication of the automated method, it has been found that the serum, which was used as both diluent and larval medium, had a tendency to sieze-up the pistons of the Auto-diluter. The serum has therefore been replaced by water as the diluent. The initial water solutions are prepared at 4 times the required concentration and bovine serum added to these at a ratio of 3:1 thus achieving the required dilutions. 5 m*l* of each solution is pipetted into a 10,0 x 2,5 cm glass tube and absorbed on 0,5 gm. cotton wool.

Approximately 30 first instar larvae were added to each tube which was then lightly stoppered with cotton wool. A susceptible laboratory strain of *Lucilia sericata* was included for comparison. It has been shown that there is no significant difference in the response of susceptible *Lucilia sericata* and *Lucilia cuprina* larvae[11] and therefore a valid comparison may be made. Racks holding the range of dilutions were left in a darkened incubator at 27°C for 72 hours.

At the end of the incubation period the larvae were assessed in one of the following categories: (i) dead – all larvae dead; (ii) affected – some larvae dead or affected in vitality, (iii) normal – all larvae actively feeding. The minimum lethal concentration (MLC) and minimum affecting concentration (MAC) for each strain and insecticide were thus obtained.

Each strain was tested against arsenic, dieldrin and diazinon. In addition the "Libertas" strain was tested against chlorfenvinphos and fenthion-ethyl.

RESULTS

The results of tests on the ten field strains, together with the corresponding susceptible strain results are given in Table 1. Figures for MLC and MAC are in parts per million (ppm). The resistant factor (RF) is also given where appropriate; this figure is obtained by dividing the MLC of the field strain by the MLC of the susceptible strain.

Table 1: RESULTS OF TESTS ON TEN STRAINS OF *L. CUPRINA* WITH ARSENIC, DIELDRIN, DIAZINON, DICHLOFENTHION AND CHLORFENVINPHOS

Strain	Date Received for Testing	Arsenic			Dieldrin		Diazinon		
		MLC	MAC	RF	MLC	MAC	MLC	MAC	RF
"CROMWELL"	14.2.73	240	75	4,3	32	<0,56	0,075	0,056	1,0
SUSCEPTIBLE		56	42		4,3	<0,56	0,075	0,056	
"BLAAUWFONTEIN"	24.2.72	32	24	–	32	<0,56	0,32	0,24	4,3
SUSCEPTIBLE		130	32		18	<0,56	0,075	<0,042	
"ELGIN"	10.3.72	42	13	–	42	<0,56	0,42	0,24	3,2
SUSCEPTIBLE		100	32		13	<0,56	0,13	0,075	
"KWANYANGA"	23.5.72	42	18	–	18	<0,56	0,75	0,56	4,2
SUSCEPTIBLE		42	18		42	<0,56	0,18	0,075	
"DONNYBROOK"	21.6.73	13	7,5	–	7,5	<0,56	0,24	0,18	4,3
SUSCEPTIBLE		56	24		32	<0,56	0,056	0,042	
"ABBOTSBURY"	8.5.72	42	24	1,3	1,8	<0,56	2,4	1,0	12,3
SUSCEPTIBLE		32	18		1,0	<0,56	0,18	0,13	
"BRAKLYN"	8.5.72	24	<7,5	–	42	<0,56	2,4	1,0	18,0
SUSCEPTIBLE		56	24		24	<0,56	0,13	0,1	
"BRANDKRAAL"	8.5.72	42	7,5	–	>42	<0,56	1,0	0,56	5,5
SUSCEPTIBLE		56	18		32	<0,56	0,18	0,13	
"LIBERTAS"	14.9.73	56	24	–	24	<0,56	1,0	0,56	13,3
SUSCEPTIBLE		100	32		32	<0,56	0,075	0,056	
"WILDEALSPUT"	28.5.72	32	<7,5	–	1,0	<0,56	1,3	0,42	–

	Dichlofenthion			Chlorfenvinphos		
	MLC	MAC	RF	MLC	MAC	RF
"LIBERTAS"	>5,6	>5,6	>23,8	1,3	1,0	40,6
	0,18	0,1		0,032	0,013	

MLC = Minimum lethal concentration in parts per million
MAC = Minimum affecting concentration in parts per million
RF = Resistance Factor

DISCUSSION

Of the ten strains tested, only the "Cromwell" strain showed resistance to arsenic (RF 4,3); this could be attributed to the prolonged use of arsenic on this property.

When assessing the results obtained with dieldrin past experience has shown that the MAC figure is more indicative of activity than the MLC; this is because dieldrin is a poor larvicide but prevents larvae from behaving in a normal manner at low levels in the wool. Hence none of the strains exhibited resistance when this criterion was used. Dieldrin was in widespread use for only about 3 years and since it is no longer available for use on sheep the problem of resistance is of little consequence.

Four of the strains, "Blaauwfontein", "Elgin", "Kwanyanga" and "Donnybrook" show a low-order resistance to diazinon which would nevertheless indicate reduced protection in the field. These four strains, together with the "Cromwell" strain all originated from the eastern half of South Africa where bodystrike is more prevalent than breech strike. Treatment is less intense and thus selection pressure on the larvae would be low.

The remaining *Lucilia cuprina* strains, "Abbotsbury", "Braklyn", "Brandkraal" and "Libertas" all show a high level of resistance to diazinon. These strains originate from an area where breech strike is more common than bodystrike. Treatment is therefore more intense and results in greater selection pressure on the feeding larvae owing to the higher concentration of insecticide used. This is supported by evidence from the field where treatment for blowfly control has increased from twice per season to six or even eight times per season in the past year in order to achieve adequate control.

It is interesting to note that the *C. albiceps* strain from "Wildealsput" also shows a high MLC to diazinon; although no susceptible reference strain is available for comparison it would appear that this strain too is resistant to diazinon. This fly is closely associated with sheep and secondary invasion by it often follows a strike initiated by *Lucilia cuprina;* it is therefore subjected to quite considerable insecticidal selection pressure. For possible resistance to develop in both *C. albiceps* and *Lucilia cuprina* is an alarming prospect.

CONCLUSION

The findings reported here foreshadow a deteriorating situation for the sheep farmers of South Africa with regard to increasing failures of fly strike preventives following the advent of o.p. resistance in the blowfly *Lucilia cuprina*.

Although the factors of resistance quoted are not as high as in some Australian strains[9], continued use of insecticides will inevitably maintain selection pressure on the field population thus aggravating the problem of blowfly resistance.

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BOOK REVIEW — RESENSIE

HISTOLOGY AND COMPARATIVE ORGANOLOGY: A TEXT-ATLAS

WILLIAM J. BANKS Ph.D.

The Williams & Wilkins Company: Baltimore 1974. pp X 285 Figs. 556 Approximate price R21,00.

Of the many text-atlasses available in histology I find this one the most suitable for veterinary students. The text is essentially a summary of the most important features which pregraduates are expected to know. It is also surprisingly complete and up to date as regards to new findings. Most of these features are also illustrated in well annotated black and white photographs or diagrammatic sketches. The photographs are mostly from sections of organs of domestic or wild life species making it all the more applicable to veterinary or biology students. Species differences and organs unique to some species as well as avian organs are discussed when appropriate.

It is a book ideally suited for purposes of revision, for self-study and as an illustrated guide in practical histology classes. It can by no means replace the standard textbook, nor was that ever the intention of the author. Ample references are given at the end of each chapter.

It is a book which I can highly recommend.

W.H.G.

ARSENIC RESISTANCE IN SPECIES OF MULTI-HOST TICKS IN THE REPUBLIC OF SOUTH AFRICA AND SWAZILAND

M.D.MATTHEWSON* AND J.A.F. BAKER**

SUMMARY

A survey was made in the Republic of South Africa and Swaziland to determine the incidence of arsenic resistance in multi-host ixodid ticks. Arsenic resistant strains were found in the following species:-
Rhipicephalus appendiculatus, Rhipicephalus evertsi, Rhipicephalus capensis, Amblyomma hebraeum and a species of *Hyalomma*, tentatively identified as *Hyalomma rufipes*.
Strains of *Haemaphysalis Leachi, Haemaphysalis silacea* and *Ixodes pilosus* were also tested.

INTRODUCTION

Ixodid ticks have shown a facility for developing resistance to chemicals introduced for their control. Resistance in the one-host boophilid ticks has been recorded to arsenic and to certain chemicals of the following groups – the chlorinated hydrocarbons, the organophosphorus esters and the carbamates. The resistance status of these ticks has been reviewed by Wharton and Roulston[8]. The two and three-host ticks, as might be expected from the greater length of their life-cycle and their broader host range, are under considerably less selection pressure from ixodicides and hence have not developed resistance to the same extent as have the one-host ticks.

However, resistance in the two-host tick *Rhipicephalus evertsi* has been recorded to toxaphene[6 9] gamma BHC[6 9] and dieldrin[9] and in the three-host ticks to chlordane[4] and to chlordane and gamma BHC[5] in *R. sanguineus* and to toxaphene and gamma BHC in *R. appendiculatus*[2 6]. This paper records the results of a survey of arsenic susceptibility carried out in the Republic of South Africa and Swaziland over a period of two years. The 126 field strains received and tested embraced nine species of multi-host ticks.

METHODS

Engorged female ticks received from field collections were identified and placed in an incubator at 24°C and 80° R.H. The susceptibility of the larval stage was tested when the larvae were 14-21 days old. The technique used is described by Shaw[7]. Larval ticks were immersed between two filter papers in a serial range of solutions of arsenic for a period of 10 minutes. The larvae were then removed from the solution, dried carefully, and a number placed in a folded filter paper packet which was then sealed to prevent escapes. The packets were placed in an incubator maintained at 24°C and 80° R.H. for a period of 72 hours when percentage mortality is assessed. Water controls were run concurrently with each test and corrections for control mortality made using Abbott's formula[1].

* Wellcome Research Laboratories, Berkhamsted, Hertfordshire, England
** Kwanyanga Research Station, Coopers (South Africa) (Pty) Limited, Greenfields, East London, Republic of South Africa

RESULTS

The results, expressed as LC50(%) values, are the product of at least two replicates for each observation. The data were analysed by computer, using a probit analysis programme, and the results have been tabulated, (Tables 1-5), for each of the nine species of multi-host ticks tested.

DISCUSSION

Arsenic was first utilised for cattle tick control in 1893[3] in the Republic of South Africa and for some fifty years was the only effective ixodicide available to the cattle farmer. The development of arsenic resistance by the Blue tick, *Boophilus decoloratus* and the discovery of other groups of chemicals, effective as ixodicides and not resisted by this tick, gradually led to the displacement of arsenic, although it is still used in parts of the Republic of South Africa and Swaziland.

In Africa mixed infestations of single and multi-host ticks are common and, given the long history of arsenic usage, resistance development might be expected in species of two and three host ticks although at a slower rate than in the one-host boophilid species. There has, however, not been any recorded resistance by multi-host ticks to arsenic.

A paper by Whitehead and Baker[9] refers to two strains of *R. evertsi* which, from laboratory and field testing, were found to be susceptible to arsenic.

The time that has elapsed since the widespread usage of arsenic, some thirty years, together with the often incomplete histories of ixodicidal usage on most properties and the movement of cattle from one area to another has made it impossible to define any one of the strains obtained from cattle as being "primitive", or entirely susceptible to arsenic. Because of this lack of an accurate base-line it has therefore not been possible to measure factors of resistance for each of the field strains.

The results, however, do show significant variations between LC50 values for the following species *R. evertsi, R. appendiculatus, R. simus* and the species of *Hyalomma*. If the lowest LC50 figure for each species is taken as the base-line and the other strains compared with it the factors of difference are in the order of 2-11 times dependent upon the species concerned. Arsenic is a hazardous chemical, both for the stock and the farmer and the concentration range recommended by the manufacturer, 0,16 – 0,32%

Table 1: THE SUSCEPTIBILITY OF LARVAE OF THE TWO-HOST TICK *RHIPICEPHALUS EVERTSI* TO ARSENIC

Field Strain Sample Number	Origin of Strain		LC50 (%)	95% Confidence Limits	
				Lower	Upper
1	East London	Eastern Cape	1,57	1,20	1,91
2	"	"	2,04	1,97	2,11
3	"	"	2,02	1,40	2,55
4	"	"	2,21	1,28	3,21
5	"	"	2,27	1,66	2,96
6	"	"	4,45	4,04	5,01
7	"	"	2,02	1,04	3,16
8	"	"	1,91	1,49	2,41
9	"	"	1,91	1,78	2,03
10	Albany	"	1,79	1,21	2,36
11	Queenstown	"	1,74	1,09	2,74
12	Potgietersrus	Northern Transvaal	1,39	1,05	1,77
13	Soutpansberg	Northern Transvaal	0,84	0,60	1,12
14	Sibasa	Venda	0,71	0,20	1,25
15	"	"	1,69	1,58	1,80
16	"	"	1,77	0,78	2,80
17	"	"	0,66	0,13	1,16
18	"	"	1,77	0,78	2,80
19	Vuwani	"	3,13	2,66	3,94
20	Ngqeleni	Transkei	1,65	0,51	2,69
21	Libode	"	2,11	1,97	2,25
22	Umzimkulu	"	1,67	1,12	2,24
23	Mqanduli	"	2,58	2,46	2,71
24	Matatiele	"	1,62	0,87	3,25
25	"	"	1,75	1,56	1,91
26	Elliotdale	"	4,12	3,47	5,54
27	Tsolo	"	1,68	1,28	2,18
28	Hhohho	Swaziland	1,19	0,17	2,55
29	"	"	2,08	1,36	2,95
30	"	"	1,24	0,78	1,73
31	"	"	1,62	1,49	1,76
32	"	"	1,11	0,68	1,84
33	Lubombo	"	1,11	0,29	1,88
34	"	"	1,25	0,93	1,59
35	Pietermaritzburg	Natal	1,21	0,45	2,09

Table 2: THE SUSCEPTIBILITY OF LARVAE OF THE THREEHOST TICK *AMBLYOMMA HEBRAEUM* TO ARSENIC

Field Strain Sample Number	Origin of Strain		LC50 (%)	95% Confidence Limits	
				Lower	Upper
1	East London	Eastern Cape	1,96	1,42	2,53
2	"	"	2,10	2,01	2,20
3	"	"	1,59	0,73	2,95
4	Port Shepstone	Natal	1,19	1,03	1,35
5	Alfred	Kwazulu	1,10	1,02	1,18
6	Pilgrim's Rest	Eastern Transvaal	2,15	1,99	2,29
7	Thabazimbi	Northern Transvaal	4,32	3,38	6,24
8	Sibasa	Venda	2,45	1,80	3,91
9	"	"	2,77	1,80	3,95
10	"	"	3,46	2,87	4,31
11	"	"	3,09	2,89	3,30
12	"	"	3,25	2,31	4,18
13	"	"	2,87	1,56	4,52
14	"	"	3,44	2,85	4,28
15	"	"	3,12	2,87	3,38
16	"	"	1,89	1,77	2,01
17	"	"	2,73	1,78	4,06
18	"	"	3,06	2,80	3,34
19	"	"	2,15	0,40	3,93
20	"	"	2,18	1,40	3,00
21	"	"	2,33	1,21	4,17
22	Vuwani	"	2,98	2,82	3,16
23	"	"	2,32	1,86	2,82
24	"	"	2,48	2,36	2,61
25	Giyani	Gazankulu	2,46	1,98	3,11
26	Malamulele	"	2,14	1,46	3,11
27	Kentani	Transkei	1,96	1,64	2,33
28	Lubombo	Swaziland	2,39	1,69	3,61
29	"	"	2,20	2,07	2,34
30	"	"	2,69	2,47	2,95
31	"	"	3,15	2,99	3,32
32	"	"	2,41	2,18	2,67
33	Hhohho	"	2,94	1,84	4,93

As_2O_3, must be strictly adhered to if accidents are to be prevented.

It can be seen that small increases in tolerance by the tick to arsenic, by the development of resistance, can lead to breakdowns in control which cannot be remedied by raising the concentration of the chemical in the dipping bath. The farmer is recommended, in this instance, to change to another ixodicide.

Comparatively high LC50 values were also found in two other species – *A. hebraeum* and *R. capensis*. Although comparisons between different species of ticks should not be drawn too closely the LC50 values for these two species are of a similar order to those of the more resistant strains in the other three species and these ticks may reasonably be typed as resistant to arsenic. The strains of *H. silacea* and *I. pilosus* can be provisionally typed as susceptible to arsenic by the same reasoning.

The strain of *H. leachi* which originated from a dog can be typed definitively as susceptible to arsenic. This tick is specific to the Canidae and is unlikely to have been exposed to selection by arsenic as this chemical is not recommended for treatment of ticks on dogs.

Table 3: THE SUSCEPTIBILITY OF LARVAE OF THE THREE-HOST TICK *RHIPICEPHALUS APPENDICULATUS* TO ARSENIC

Field Strain Sample Number	Origin of Strain		LC50 (%)	95% Confidence Limits	
				Lower	Upper
1	East London	Eastern Cape	2,30	1,73	3,17
2	"	"	1,68	1,42	1,95
3	"	"	1,67	0,87	2,18
4	"	"	2,42	1,90	2,94
5	"	"	1,53	0,94	2,24
6	"	"	1,03	0,93	1,13
7	Vryheid	Natal	1,56	1,42	1,70
8	Port Shepstone	Kwazulu	1,36	1,23	1,48
9	Alfred	Kwazulu	1,54	0,57	2,60
10	Ngqeleni	Transkei	2,08	1,95	2,20
11	"	"	1,16	1,04	1,27
12	"	"	1,93	1,51	2,40
13	"	"	1,15	0,67	1,67
14	"	"	1,50	1,38	1,64
15	Umtata	"	2,23	1,57	3,40
16	Kentani	"	2,19	2,07	2,31
17	Libode	"	2,07	1,37	2,86
18	"	"	2,60	1,45	4,93
19	"	"	1,93	1,28	2,83
20	Hhohho	Swaziland	1,84	1,73	1,95
21	"	"	1,58	1,48	1,68
22	"	"	3,78	3,32	4,42
23	"	"	2,30	2,17	2,43
24	Lubombo	"	1,66	0,50	3,66
25	Sibasa	Venda	0,67	0,41	0,91
26	"	"	0,32	0,22	0,41
27	"	"	0,57	0,52	0,61
28	"	"	0,70	0,19	1,19
29	"	"	0,68	0,64	0,72
30	Vuwani	"	1,83	1,02	3,09
31	Potgietersrus	Northern Transvaal	0,98	0,61	1,43

Table 4: THE SUSCEPTIBILITY TO ARSENIC OF LARVAE OF A SPECIES OF *HYALOMMA* TENTATIVELY IDENTIFIED AS *H. RUFIPES*

Field Strain Sample Number	Origin of Strain		LC50 (%)	95% Confidence Limits	
				Lower	Upper
1	Queenstown	Eastern Cape	2,28	1,90	2,74
2	Kuruman	Northern Cape	2,93	2,16	4,22
3	Herschel	Ciskei	2,72	2,02	4,02
4	Vryheid	Natal	1,68	1,52	1,86
5	Pietermaritzburg	"	7,57	6,13	10,24
6	Sibasa	Venda	2,58	1,83	3,56
7	"	"	1,52	1,39	1,65
8	"	"	2,40	2,22	2,60
9	"	"	2,59	1,92	4,19
10	Potgietersrus	Northern Transvaal	1,77	1,62	1,92
11	Soutpansberg	Northern Transvaal	1,73	1,37	2,27
12	Zastron	Orange Free State	2,96	1,59	5,21

CONCLUSIONS

The variation in response shown by tick larvae of a number of strains within six of the species tested are indicative of resistance to arsenic.

These species include *Rhipicephalus evertsi, R. appendiculatus, R. simus, R. capensis, Amblyomma hebraeum* and a species of *Hyalomma* tentatively identified as *H. rufipes*. The single strains of *Haemaphysalis leachi, H. silacea* and *Ixodes pilosus* tested were considered to be fully susceptible to arsenic.

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Table 5: THE SUSCEPTIBILITY OF VARIOUS TICK SPECIES TO ARSENIC

Tick Species	Field Strain Number	Origin of Strain		LC50(%)	95% Confidence Limits	
					Lower	Upper
Rhipicephalus simus	1	East London	Eastern Cape	1,30	0,76	1,91
"	2	"	"	1,90	0,94	3,01
"	3	"	"	1,42	0,97	1,83
"	4	"	"	2,19	1,81	2,65
"	5	Fort Beaufort	"	1,88	1,30	2,90
"	6	Mdantsane	Ciskei	1,82	1,73	1,91
"	7	Umzinto	Kwazulu	0,84	0,78	0,89
Rhipicephalus capensis	1	Victoria East	Ciskei	1,57	1,45	1,67
"	2	Libode	Transkei	1,49	1,10	1,92
"	3	"	"	2,05	0,89	3,96
"	4	Elliotdale	"	1,08	0,99	1,16
"	5	Hhohho	Swaziland	1,10	1,05	1,16
Ixodes pilosus	1	East London	Eastern Cape	0,43	0,26	0,69
Haemaphysalis leachii	1	"	"	0,37	0,33	0,40
Haemaphysalis silacea	1	"	"	0,76	0,58	1,08

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REPRODUCTIVE CAPACITY AND LONGEVITY OF STABLE FLIES MAINTAINED ON DIFFERENT KINDS OF BLOOD

G.D.G.DU TOIT*

SUMMARY

An investigation was made into some lesser known aspects of the biology of the stable fly, *Stomoxys calcitrans*, in order to find a basis for possible means of natural control of the pest on dairy farms.

The reaction of adults to different kinds of blood was tested. Diets investigated were bovine blood alternated with ovine blood, bovine blood alternated with pig's blood, bovine blood alternated with manure filtrate, bovine blood alone, ovine blood alone, pig's blood alone and manure filtrate alone.

Adults could not servive on manure filtrate alone. Flies in the other treatments exhibited no marked differences as regards duration of pre-mating-, pre-oviposition- and oviposition periods. There were, however, differences in the number of eggs laid. Females maintained on bovine blood alone produced most eggs i.e. 2,3 times as many as those laid by females fed on pig's blood alone. The latter were the poorest producers. Viability and incubation period of eggs did not appear to be affected by the kind of blood fed to the adults. Flies, however, lived longer when supplied with bovine blood and died sooner when maintained on pig's blood.

INTRODUCTION

Stomoxys calcitrans, the stable fly, is a world-wide pest which also occurs abundantly in the Eastern parts of South Africa where summer conditions are favourable for its existence. It attacks practically all domestic animals but is of special economic importance on dairy farms where, as stated in the Merck Veterinary Journal[1], milk production may decrease as much as an estimated 50 per cent and body mass losses of 10 to 15 per cent in livestock may occur during severe attacks of the pest. It has been proved experimentally that this fly can act as a mechanical vector of certain animal diseases[3 5].

Despite the fact that scientific publications report on the stable fly from time to time, no satisfactory program for its control in South Africa exists. In a recent paper on Diptera, Nevill[6] discusses the importance of the *Stomoxydinae* in Southern Africa and clearly illustrates the need for further research on the biology of *S. calcitrans* and methods for its control.

Whilst the use of insecticides should not be summarily condemned, there is considerable merit in pursuing the modern trend in pest control which tends to exclude the use of hazardous chemicals. Accordingly it was decided to investigate those aspects of the biology of *S. calcitrans* that could probably be exploited for the purpose of finding more effective methods of natural control.

Du Toit[2] determined the essential requirements for the larvae. The effect on larvae of eight different breeding media, that could occur naturally on a dairy farm was investigated. Where larvae were reared under conditions of overcrowding or in media of poor quality, a smaller number reached the pupal stage; the pupae were of sub-normal mass and gave rise to adults producing fewer eggs than normal.

As with the requirements of the immature stages, it is believed that a knowledge of adult life requirements is essential before a programme of natural control measures can be devised. Information on the effect which the kind of blood available to the adult has on reproduction and longevity is scanty.

The only food source of adult *S. calcitrans* is mammalian blood, and it is imperative for them to have this if they are to reproduce. It is therefore important to establish which of the kinds of blood normally available to them are most conducive to reproduction and longevity. Jones[4] states that the only satisfactory diet is blood of such mammals as cattle, horses and pigs whereas, according to Pospisil[7] female *Stomoxys* do not lay eggs when fed on the blood of sheep, guinea-pigs or mice. In a study on the mosquito *Culex salinarius*, Shelton[8] found that not only the source but also the size of the blood meal was important for egg production. Tuttle[9] undertook a study on the survival and oviposition habits of *S. calcitrans* on various diets. He examined the factors present in the red cell fraction of bovine blood which influence oviposition.

Certain management practices can be carried out on dairy farms where the opportunities for stable flies to obtain bovine blood can be reduced while animals, of which the blood is less suitable, are made freely available for them to feed on. In order to find a basis for such managemental practices, it was decided to investigate the effect which some of the naturally occurring adult diets had on the reproductive capacity and longevity of the fly.

MATERIALS AND METHODS

Flies from an established colony were used.

A variety of warm-blooded animals may be found on a dairy farm, thus the suitability of bovine-, ovine- and pig's blood and combinations thereof were examined as food for adult *S. calcitrans*.

Statistical design: A simple random design was used where the seven different diets were taken as different treatments with 20 flies (10 males and 10 females) for each as individual replicates. Significance tests of treatment means were done against established population means.

Treatments: The following adult diets were investigated:

Citrated bovine blood only;
Citrated ovine blood only;

* Agricultural Research Institute, Eastern Cape Region, Dohne.

Citrated pig's blood only;
Manure filtrate only;
Citrated bovine blood alternated with citrated ovine blood;
Citrated bovine blood alternated with citrated pig's blood;
Citrated bovine blood alternated with manure filtrate;

Each diet was regarded as a separate treatment.

Experimental design: Seven cages, each containing twenty newly emerged flies (10 male and 10 female) taken from a stock colony were placed in a temperature/humidity controlled room. They were supplied once daily with cottonwool pads soaked in the adult food media listed above. Where two kinds of food were used (e.g. bovine blood/ovine blood), these were alternated on a daily basis.

The flies were examined daily. As soon as mating started each cage was supplied with an oviposition dish[2].

The following data were recorded:
Time taken for flies to start mating;
Time taken for flies to start laying eggs;
Number of eggs laid each day per oviposition dish;
Duration of egg-laying period;
Viability of eggs;
Lifespan of flies.

RESULTS

Duration of pre-mating period: The first matings were observed in the cage supplied with ovine blood. Flies were four and a half days old at this stage. In all other cages except for that supplied only with manure filtrate, first matings took place when the flies were five to six days old.

Duration of pre-oviposition period: First eggs were laid by females in the cages supplied with bovine-, pig's- and bovine-/ovine blood. Flies were six and a half days old at this stage. In the cages supplied with ovine-, bovine-/pig's- and bovine blood/manure filtrate, first eggs were laid when the flies were seven and a half days old.

Number of eggs laid: The mean numbers of eggs laid during their lives by females in the different treatments are given in Table 1.

Table 1: MEAN NUMBERS AND THE VIABILITY OF EGGS LAID BY FEMALE FLIES

Treatment	No of eggs laid	Viability of 25 eggs	
		No hatching	% hatching
Bovine/ovine blood	262,1	23	92
Bovine/pig's blood	181,4	24	96
Bovine blood/manure filtrate	218,5	22	88
Bovine blood	372,2	24	96
Ovine blood	270,5	20	80
Pig's blood	160,8	23	92
Manure filtrate	0,0	–	–

Duration of oviposition period: The period of active oviposition of any female, regardless of treatment, was found to last from the onset of egg laying until death.

No information is available on the frequency with which a female laid her consecutive batches of eggs as the method of recording did not allow for this information to be obtained.

Vaibility of eggs: Tests on viability of the eggs revealed no marked differences amongst the different treatments. The number of eggs out of 25 hatching in each treatment is given in Table 1.

Incubation period of eggs: In none of the treatments did the incubation period of the eggs differ markedly from the colony mean of one and a half days. Any differences which may have occurred were so small as to have been undetectable by the methods of observation employed.

Lifespan of flies: Mortalities amongst flies in the different treatments are graphically illustrated in Fig. 1. The period of nil mortality was longest in the ovine blood treatment where the first flies died only after they were 11 days old. The time which elapsed before 50 per cent mortality had occurred was much the same for all treatments except manure filtrate. The longest lifespan was recorded in flies fed on bovine blood where 100 per cent mortality was only recorded after 43 days.

In order to obtain a clearer comparison of total lifespan, the number of "fly days" for each treatment was calcluated by adding the number of flies alive in each cage every day until the last one had died.

The duration of the nil mortality, 50 per cent mortality and 100 per cent mortality periods and total number of "fly days" for each treatment is given in Table 2.

Table 2: THE DURATION OF THE NIL MORTALITY, 50% MORTALITY, 100% MORTALITY AND TOTAL "FLY DAY" PERIODS

Treatment	Duration of periods in days			
	Nil mortality	50% mortality	100% mortality	Total "Fly days"
Bovine blood	7	14	43	374
Ovine blood	11	16	36	336
Pig's blood	1	14	31	230
Manure filtrate	<1	2	2	33
Bovine- ovine blood	6	14	36	302
Bovine- pig's blood	6	13	32	281
Bovine blood/ Manure filtrate	9	16	29	390

DISCUSSION

The main objective of the present investigation was to obtain a better understanding of those aspects of the biology of *Stomoxys calcitrans* which could be exploited for the purpose of a control program of the pest in dairy herds.

The results obtained revealed no marked differences in the length of pre-mating and pre-oviposition periods of flies maintained on different diets. This, however, was not the case with the number of eggs laid. Flies fed on bovine blood produced more eggs during their life than flies in any of the other

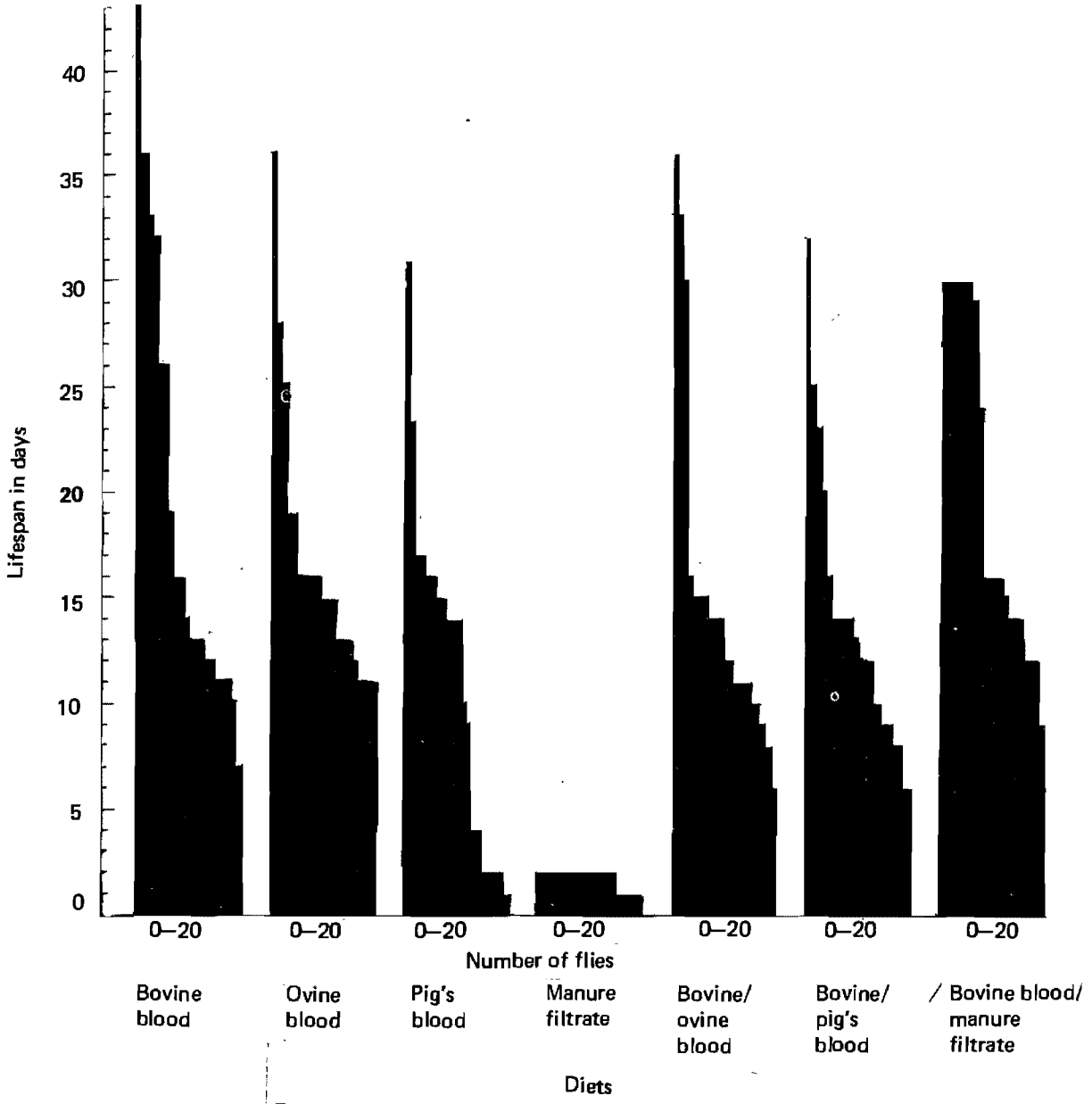

Fig. 1 Lifespan in days of flies in groups of twenty on different diets.

treatments, e.g. 2,3 times as many as produced by flies fed on pig's blood. Flies on a diet of bovine blood alternated with pig's blood laid 2,1 times fewer eggs than flies fed on bovine blood alone.

Although these results strongly indicate that pig's blood has a depressing effect on egg production, the experimental methods employed did not allow for sufficient data for statistical analysis to be obtained. Further research on adult reaction to different types of blood meal is indicated and more types, including the blood of horses, goats, poultry and even the more abundant species of game should be investigated.

Contrary to the findings of Pospisil[7], ovine blood was found to be a satisfactory diet enabling *S. calcitrans* to produce a reasonable number of eggs.

The viability and incubation period of eggs appear to be unaffected by the different diets. Lifespan, however, expressed as "fly days" showed a marked difference between flies fed on pig's blood and those on a diet of bovine blood; the latter lived considerably longer. These results, together with the information obtained on egg production shows that *S. calcitrans* is better adapted to live on the blood of cattle than on that of sheep and particularly of swine.

ACKNOWLEDGEMENTS

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EFFECTIVITY OF REV 1 VACCINE IN RAMS AGAINST BRUCELLA OVIS INFECTION

D.V. GRADWELL* AND F.E. VAN ZYL**

SUMMARY

Vaccination with Rev 1 at four months of age protected rams against experimental infection with *Brucella ovis*. A double vaccination did not improve immunity whereas rams vaccinated later in life showed a decreased immunity to the infection. Lesions and complement fixation reactions were found to be inadequate criteria for the diagnosis of the disease in the individual ram.

INTRODUCTION

From an evaluation of complement fixing (CF) antibody titres against *B. ovis* in rams in South Africa[8] it was found that vaccinated flocks showed an incidence of 3,6% infection as opposed to 5,0% infection in the non-vaccinated. This small difference was nevertheless distrubing because in previous experiments[4] Rev 1 vaccination was very effective against *B. ovis* infection in South Africa, and there was some concern that some change had occurred in the routine production of Rev 1 at Onderstepoort. With this survey[8] in mind we decided to retest the efficacy of Rev 1 vaccination as a protective measure against *B. ovis* infection even though it was stated in the survey report that the high rate of infection in vaccinated flocks could be due to faulty vaccination procedures. After this retest was started, however, it was reported[5] that infection with *B. ovis* in flocks where Rev 1 was meticulously carried out, was 0,3% whereas in flocks unreliably vaccinated and not vaccinated the figures were 18,1% and 3,4% respectively. The high rate of infection for rams on farms where unreliable vaccination practices were carried out was attributed to recent vaccination of adult rams or frequent vaccination of rams which may cause positive reaction to *B. ovis* with the CF test[7].

MATERIALS AND METHODS

Experimental Animals: Twenty 2-year old Merino rams and sixteen 14 months old Namaqua-Dorper rams were obtained from two different areas in the Cape Province. The Namaqua-Dorper rams had not been vaccinated with Rev 1 while the Merino rams had been vaccinated at four months of age.

The rams were divided into four groups:
A – Vaccinated at 14 months of age and challenged at 20 months.
B – Vaccinated at 4*** and 24 months of age and challenged at 30 months.
C – Non vaccinated and challenged at 20 months.
D – Vaccinated at 4*** months and challenged at 30 months

Complement Fixation. Sera from the rams were tested against *B. ovis* and *Brucella abortus* antigens by the CF test using the method of Worthington and Mülders[6] except that the antigens were prepared by sonification of cell suspensions with a Branson sonifier for 30 minutes. The clear supernatants were used as antigens.

B. abortus titrations were taken to a serum dilution of 1/1 280 whereas *B. ovis* titrations were only taken up to a serum dilution of 1/80 with each test.

Semen Examination. With the ram in lateral recumbency, semen was collected aseptically by electro-ejaculation, and the epididymes and testes palpated immediately thereafter.

From each semen sample a smear was made, dried in air, fixed briefly in a flame and stained by a modified Ziehl-Neelsen technique[3]. Smears were examined for *B. ovis* colonies and neutrophils.

Material from each semen sample was smeared on blood tryptose agar and incubated at 37°C for three days in an atmosphere containing 10-15% carbon dioxide. Cultures and smears were made within two hours of collecting the semen.

Colonies resembling control *B. ovis* were examined by a direct fluorescent antibody technique using a conjugate prepared from a very high titre serum as described by Nowotny[2] and then made highly specific by obsorption with sheep liver powder. Only cultures giving intensity of fluorescence equal to that of the control known *B. ovis* culture were taken as positive for *B. ovis*.

Immunisation of Rams. Half of the rams, eight Dorper-Namaqua and 10 Merinos were vaccinated subcutaneously with two ml of Rev 1 vaccine (Onderstepoort Batch R510) taken at random from the vaccine stock. Examination of this vaccine showed that each ram received 14×10^{10} colony-forming units of the Rev 1 strain.

Challenge Procedure. All rams were challenged 25 weeks after vaccination by the intravenous injection of 10 ml and intrapraeputial instillation of one ml of a three-days old culture of *B. ovis* (strain 6010) grown in liquid medium[1] and adjusted to 0,3% packed cell volume; this was found to contain, $1{,}03 \times 10^{10}$ colony-forming units of *B. ovis* per ml of medium.

RESULTS

Before starting the experiment it was established that all rams were serologically negative and *B. ovis* was not isolated from the semen on either of two occasions.

* State Veterinarian, Veterinary Research Centre, Skukuza 1350
** Dept. of Reproduction, Veterinary Research Institute, Onderstepoort 0110
*** % Vaccinated by the Chief State Veterinarian at Middelburg, Cape: no Brucella counts were done on the vaccine.

The infection rates after challenge are shown in Table 1.

In all but two cases rams which reached to challenge were found to be positive on both smear and

Table 1: INFECTION RATES IN THE RAMS AFTER CHALLENGE

Group	No. in the Group	Vaccinal Status	No. Positive on semen smear	No. Positive on semen cult.	No. Positive on clinical lesions	No. at the conclusion of Experiment	No. infected Total no.
A	8	Vacc. 14m	3	3	1	8*	3/8
B	10	Vacc. 4 and 24m	1	1	1	7	1/10
C	8	No. Vacc.	5	5	2	7	5/8
D	10	Vacc. 4m	0	0	0	9	0/10

* = Five rams died from unrelated causes after challenge and *B. ovis* was not isolated from any organs before or after death.

No breakdown occured in the Group D rams which were vaccinated by the recommended method despite a very heavy challenge dose more than two years later. In Group B with a double vaccination one ram out of 10 became infected. There were three breakdowns among the eight Group A rams that were immunized once, when they were 14 months old. This group, however, fared better than the control Group C where five out of eight rams became infected. The groups are too small to permit definite conclusions, and a breed difference may also have played a role as eight of the nine infected rams were Namaqua-Dorpers and only one Merino.

culture from the same semen sample. From one ram *B. ovis* was cultured but not detected in the smear and in another the smear was positive but the culture overgrown with fungus.

Lesions were palpable in only four of the nine rams excreting *B. ovis*.

The results of the serological tests are shown in Fig. 1. A geometric mean was taken of the reciprocals of the titres of the sera of the rams in each group and the graph plotted. Immediately after vacination antibody titres to *B. abortus* antigen rose sharply and these rose higher and remained high for a longer period in the rams that had received a double vaccination (Group B) than in those that had received a single vaccination (Group A). A similar picture was

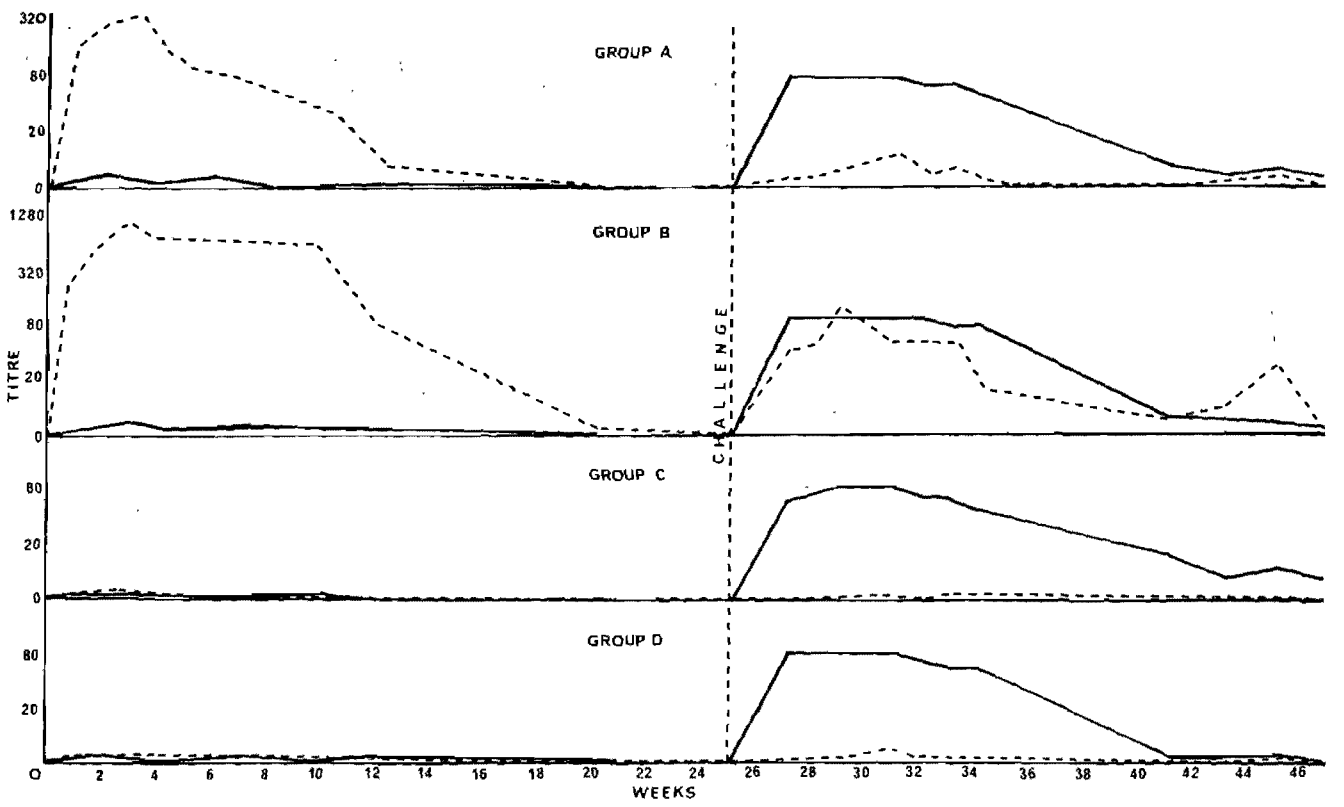

Fig 1: AVERAGE OF THE RECPIROCALS OF THE GEOMETRIC MEANS OF THE TITRES IN EACH GROUP

– – – – – – = B. abortus titres ——— = B. ovis titres

seen in these two groups as regards the antibody titres to *B. obortus* after challenge with virulent *B. ovis* organisms. Again all the rams in Group B had antibody titres to *B. abortus* antigen of greater than 1/20 which is the level taken in this laboratory indicative of positive reactors while only four rams become positive again in Group A. All rams were serologically negative to both antigens six months after vaccination.

Rams vaccinated at weaning age (Group D) had very low antibody titres to *B. abortus* and in Group C they were negligible. No ram in these two groups had at any stage a titre of 1/20 or more to *B. abortus* before or after challenge with *B. ovis*.

In Group A, antibody titres to *B. ovis* after vaccination became positive in three rams on one occasion each at the first or second week, but in Group B five rams showed positive antibody titres after vaccination, two of which were positive a number of times up until ten weeks after vaccination. Groups C and D had negative antibody titres to *B. ovis* up to the time of challenge. All rams showed positive antibody titres to *B. ovis* antigen after challenge but by the termination of the experiment three rams from Group A, three from Group B, three from Group C and none from Group D were still showing positive titres. (Table 2)

Table 2: COMPLEMENT FIXING ANTIBODIES IN RAMS AFTER CHALLENGE

Group	Serologically +ve at end of Experiment	Serol. +ve Excretors	Serol. +ve non Excretors	Serol. −ve Excretors
A	3	2	1	1
B	3	1	2	0
C	3	3	0	2
D	0	0	0	0

In Group A the serum of one ram which was excreting organisms contained no detectable antibody whereas in one ram in which no organisms were found in the semen, the CF antibody titre remained positive throughout. Two rams from Group B also showed positive antibody titres but no organisms could be found in their semen, while in Group C two rams with no antibody were excreting organisms.

Group D rams remained bacteriologically negative and were all serologically negative at the termination of the experiment.

DISCUSSION

Vaccination of rams with Rev 1 against *B. ovis* infection at weaning age was effective (no infection in nine rams). A double vaccination does not improve the immunity (one infected ram out of seven) but complicates the serological diagnosis of the disease for up to six months after the second vaccination. Further vaccination could make serological interpretation of the CF test impossible. It is therefore definitely inadvisable to vaccinate rams annually with Rev 1 strain. Single vaccination of adult rams did not give a very high rate of protection (three infected rams out of seven), but in this experiment a very severe challenge dose was used. In the field adult vaccination may well give some protection against the usually smaller *B. ovis* challenge, and in an outbreak of *B. ovis* infection in a flock it may well be justifiable to vaccinate all unvaccinated rams. Serologically this adult vaccination will only give diagnostic problems for a few weeks after vaccination. However, in areas where *B. melitensis* infection occurs, adult vaccination should not be used at all. This is the case especially in South West Africa where Karakul rams may become infected with *B. melitensis* but a titre to *B. abortus* might be interpreted as resulting from Rev 1 vaccination if adult vaccination is practised on a large scale.

As far as the diagnosis of *B. ovis* infection is concerned the examination of semen gave reliable results in this experiment. In the single instance where the organisms were cultured but not seen on smears, this occurred only during the first after infection; in all later collections organisms were found in the smears. On these grounds it may be stated that careful examination of smears from two different lots of semen, collected at least one week apart, would pick out nearly all genitally-infected rams. Bacteriological culture of the semen therefore only helped to confirm the diagnosis.

Palpation of the testes was of little value in diagnosis as less than 50% of the infected rams showed lesions. However it may be used in flocks to indicate whether *B. ovis* infection exists.

CF test identified six of the nine excretors only and three rams that were excreting organisms would not have been detected on serologic[illegible] examination alone. The reason for this is not known and suggests that our CF test should be used only as a flock test. From three serologically positive rams no organisms were isolated from their semen. These three rams were in Groups A and B and may have become negative later, since in these groups the rams were vaccinated as adults and this could be the reason for persistent titres to *B. ovis*. Alternatively the rams may not have been genitally infected and the infection localised elsewhere. Had these rams been genitally infected the organisms should have been cultured from the testes, epididymes, ampullae or vesicles after slaughter but this was not accomplished.

If it is accepted that these three rams were infected somewhere other than in the genitalia then no false positives were obtained with the CF test and therefore any ram showing a titre to *B. ovis*, if not recently or repeatedly vaccinated with Rev 1 vaccine, can be considered to be infected with *B. ovis*. However, the CF test does not detect all infected rams in a flock.

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ASPECTS OF FORCED EXERCISE AND THE THERAPY THEREOF IN SHEEP

MARGARETHA D. GERICKE* AND P.C. BELONJE**

SUMMARY

Sixteen Merino sheep were subjected to forced exercise after which half were treated with sodium bicarbonate, vitamin B complex, antibiotics and a glucocorticoid. Cardiac rate, rectal temperature and blood were taken from all the animals before, immediately after and again two and five hours after exercise.

Exercise resulted in inreased cardiac rate, rectal temperature, plasma levels of glucose, lactate, lactate dehydrogenase, creatine kinase, potassium, and decreased levels of blood pH, buffer base, bicarbonate, base excess and CO_2.

Compared with the controls, the therapy increased plasma glucose and blood pH, buffer base, bicarbonate and base excess, while there was a significant decrease in plasma potassium. This latter effect may be important in the therapy of capture myopathy in wild animals as hyperkalaemia may be one of the causes of death in the syndrome[3].

INTRODUCTION

In a series of experiments with springbok[3] it was shown that capture stress resulted in a dramatic increase in plasma levels of glucose, creatine kinase, lactate dehydrogenase, lactate, myoglobin, potassium and packed cell volume with a concurrent reduction in blood pH. It was concluded that this stress resulted in a metabolic acidosis as well as increased cell membrane permeability.

As capture myopathy results in alarmingly high losses in springbok and as sheep are often stressed during transport, an attempt was made to develop a rational thereapy for the conditions by studying the effects of a combination of drugs on sheep subjected to forced exercise. In order to achieve this sheep were subjected to forced exercise and then treated with sodium bicarbonate, antibiotics, vitamin B complex and a glucocorticoid. This combination was selected to combat acidosis, prevent bacterial invasion and as general supportive therapy.

MATERIALS AND METHODS

Sixteen two-year old Merino sheep were subjected to forced exercise on the Welgevallen Experimental Farm in Stellenbosch

Before forced exercise, heart rate and rectal temperature were determined and the animals were bled. The blood was centrifuged for plasma which was stored at -20°C until analysed. A subsample of blood was taken anaerobically and kept on ice for blood gas analyses.

The animals were then chased up and down an enclosed roadway for 15-20 minutes. Directly after (stress sample) and again after two and five hours after the exercise they were examined and bled.

After the stress samples had been taken, the animals were divided at random into two groups. The one group received no treatment and the other the following:

(a) An intramuscular injection of 2 ml of a vitamin B complex (Bejectal, Abbott Laboratories);
(b) An intraperitoneal administration of 1 litre 1,4% sodium bicarbonate with 15 mg dexamethazone (Opticortenol, Ciba-Geigy) and 500 mg streptomycin sulphate, 300 mg procaine penicillin and 60 mg sodium penicillin (Diplostrecil, Novo-laboratories).

Boehringer test kits and Eppendorf photometer model 1101 M were used for the determination of plasma glucose, lactate, creatine phosphokinase (C.P.K.) and lactic dehydrogenase (L.D.H.). Plasma sodium and potassium concentrations were determined by standard flame photometric procedures (Instrumentation Laboratory, IL 343). Blood pH, buffer base, base excess, pCO_2 and plasma standard bicarbonate were determinded within 15 minutes of collection on the anaerobic subsample by means of a Radiometer Blood Micro System (BMS 2) at 37°C. In order to correct for the low oxygen saturation of venous blood a correction factor of 1,5 was added to the buffer base and base excess curves on the Siggaard-Andersen nomogram before reading base excess and pCO_2. This factor assumed that in all the sheep the haemoglobin concentration was 10g% and the O_2 saturation 50%.

Packed cell volume was determined by means of an Ecco microhaematocrit centrifuge.

RESULTS AND DISCUSSION

Cardiac rate, rectal temperature and packed cell volume (P.C.V.). Forced exercise had the expected effect on the cardiac rate and rectal temperature. As can be seen from Table 1, the values after stress were much higher than the control values. Two hours later they returned to normal. However, one animal still had a very high cardiac rate (248 beats/min.) after two hours. This animal also had the lowest plasma bicarbonate and pCO_2 and the highest lactate values after exercise. It thus seems that this animal was stressed more than the others. Morehouse and Miller (1967)[7] state that the time required for the heart rate return to normal after exercise, depends on the work load of the exercise period and the physical condition of the subject. At the five hour examination the cardiac rate of this animal had also returned to normal.

The rise in rectal temperature during exercise also represented a normal physiological result of the higher heat production during exercise.

Table 1 indicates that the P.C.V. showed no significant increase after exercise stress. The 2 h values of both the treated and untreated groups were signifi-

* Dept. Zoology and
** Dept. Animal Physiology, University, Stellenbosch, Stellenbosch 7600

cantly lower than the stressed values. This can possibly be ascribed to the fact that the animals had free access to water after exercise. If they drank enough water, the blood would have been sufficiently diluted to cause the fall in the P.C.V. Hofmeyr, Louw & du Preez (1973)[6] also found a significant increase in the cardiac rate and rectal temperature of chased zebra. In contrast with this study, they also found a highly significant increase in the P.C.V. after chasing. The increase in P.C.V. in the zebra is due possibly to the more intense alarm reaction displayed by these animals.

The results in this experiment therefore indicate a normal physiological adjustment to exercise.

Plasma levels of glucose and lactate. There was a highly significant increase in both the glucose and lactate levels of the plasma directly after exercise (Fig. 1 and Table 1). Within two hours these values returned more or less to normal. The treatment definitely affected the plasma glucose. Although there was no significant difference between the treated and untreated group at the two hour sampling, the mean of the treated group was higher. Five hours later, however, a highly significant difference existed between the two means. This could possibly be ascribed to the effect of the glucocorticoid hormone.

There was no significant difference between the 2 h and 5 h lactate values, and the treatment also had no significant effect. In other words, within two hours the animals were able to get rid of the excess lactate produced during exercise.

In this study it therefore appears that the sheep were sufficiently stressed to elicit an adrenalin response, resulting in increased glycogenolysis. Moreover, the glycogenolytic process in the muscles surpassed the mitochondrial oxidative capacity and relative muscle hypoxia resulted in elevated plasma lactate levels.

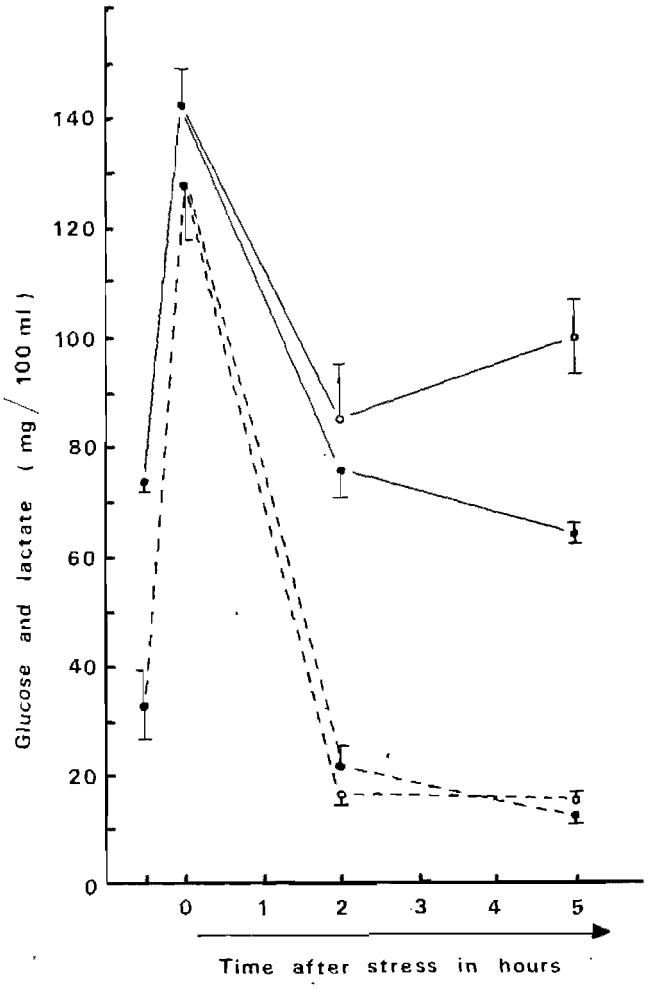

Fig. 1: The effect of forced exercise and subsequent treatment on plasma levels of glucose and lactate
Glucose ○ Treated group (± S.E.)
- Lactate ● Untreated group (± S.E.)

Table 1: THE EFFECT OF FORCED EXERCISE (STRESS) AND TREATMENT ON CARDIAC RATE, RECTAL TEMPERATURE, P.C.V., AND PLASMA GLUCOSE, LACTATE, L.D.H. AND C.P.K. OF SHEEP (MEAN ± S.D.)

Parameter	Control	After stress	2 Hours after stress		5 Hours after stress		Level of significance
			Treated	Untreated	Treated	Untreated	
Cardiac rate/min	a 127,7±29,3	abc 206,6±23,2	b 133,7±20,9	c 149,3±51,9	129,2±17,8	177,5±21,8	ab : p 0,001 c : p 0,005
Rectal temperature (C)	a 39,4±0,6	abc 41,6±0,6	b 39,4±0,3	c 39,4±0,2	39,5±0,2	39,5±0,5	abc : p 0,001
Packed cell volume (%)	32,2±2,5	ab 33,2±2,0	a 30,8±2,6	b 30,8±1,9	31,1±2,7	30,5±1,8	a : p 0,05 b : p 0,025
Plasma glucose (mg%)	afg 73,0±6,2	abc 141,6±27,3	b 84,7±23,8	cd 76,4±10,9	ef 99,1±19,0	deg 64,3±5,5	abcef : p 0,001 g : p 0,005 d : p 0,025
Plasma lactate (mg%)	a 32,8±24,9	abc 128,4±37,2	b 16,4±4,7	c 20,1±8,5	15,0±4,0	13,9±4,6	abc : p 0,001
Plasma L.D.H. (mU/ml)	456,3±57,3	a 477,3±53,0	a 547,2±44,7	505,1±62,5	572,3±54,9	509,3±69,4	a : p 0,01
Plasma C.P.K. (mU/ml)	a 34,9±15,3	abc 67,1±21,4	b 171,9±63,9	c 102,4±53,2	115,9±68,9	96,1±93,8	ab : p 0,001 c : p 0,05

Values with the *same* superscript differ significantly from one another — the level of this significance is presented in the last column.

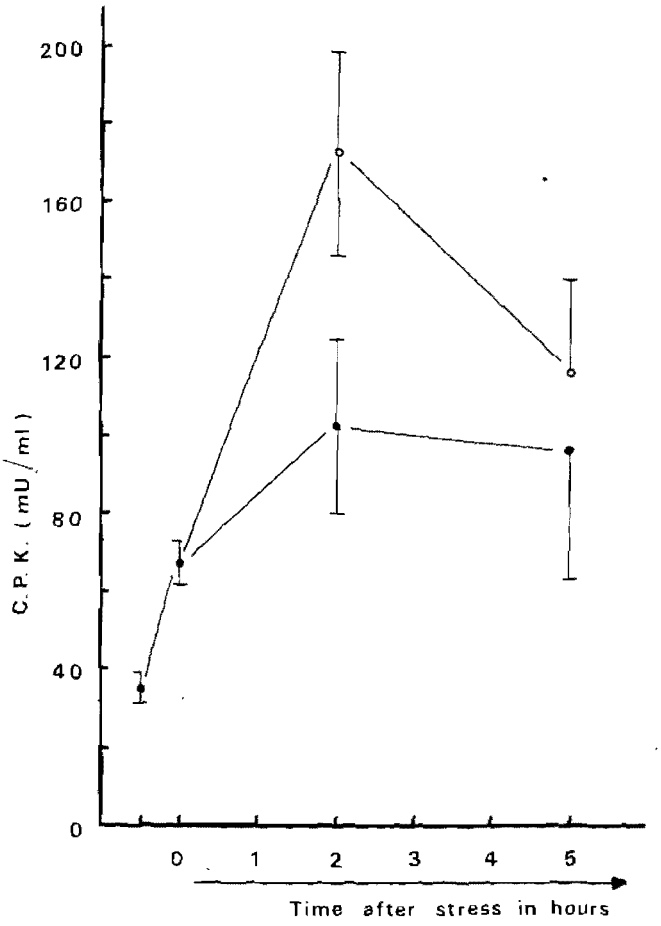

Fig. 2: The effect of forced exercise and subsequent treatment on the plasma levels of creatine kinase (C.P.K.)
○ Treated group (± S.E.)
● Untreated group (± S.E.)

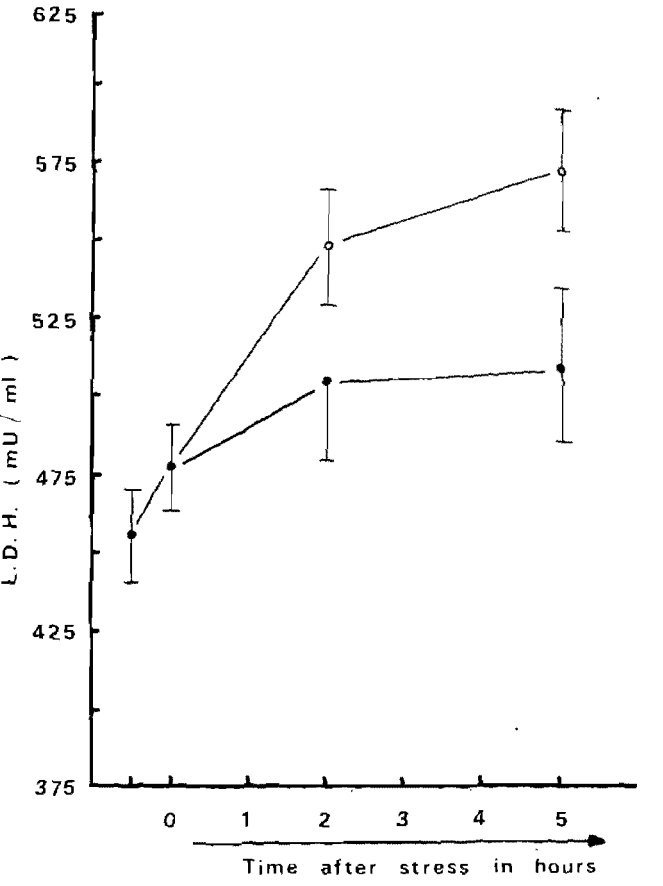

Fig. 3: The effect of forced exercise and subsequent treatment on the plasma levels of lactate dehydrogenase (L.D.H.)
○ Treated group (± S.E.)
● Untreated group (± S.E.)

Table 2: THE EFFECT OF FORCED EXERCISE (STRESS) AND TREATMENT ON PLASMA POTASSIUM AND SODIUM AND BLOOD pH, BUFFER BASE, STANDARD BICARBONATE, BASE EXCESS AND pCO_2 OF SHEEP (MEAN ± S.D.)

Parameter	Control	After stress	2 Hours after stress		5 Hours after stress		Level of significance
			Treated	Untreated	Treated	Untreated	
Plasma potassium (mEq/ℓ)	af 4,5±0,2	ab 5,1±0,7	bcdf 3,8±0,2	c 4,4±0,2	de 3,3±0,4	e 4,7±0,4	abcef : p 0,001 d : p 0,05
Plasma sodium (mEq/ℓ)	148,0±3,1	147,7±2,9	148,0±2,8	147,8±2,2	150,0±2,6	151,2±7,8	
Blood pH	aef 7,36±0,04	abc 7,28±0,08	bde 7,42±0,01	cd 7,37±0,02	f 7,43±0,05	 7,39±0,03	bd : p 0,001 aef : p 0,005 c : p 0,025
Buffer base (mEq/ℓ)	a 45,0±4,5	abc 34,9±5,1	bd 48,3±4,4	cd 43,0±1,6	 46,5±2,8	 43,7±1,9	ab : p 0,001 c : p 0,005 d : p 0,025
Standard bicarbonate (mEq/ℓ)	af 22,2±2,4	abc 16,0±3,3	bdf 26,4±1,9	cd 22,4±0,8	e 24,4±1,7	e 21,5±1,3	abcd : p 0,001 f : p 0,005 e : p 0,01
Base excess (mEq/ℓ)	af − 1,1±3,0	abc − 9,3±4,7	bdf 3,9±2,1	ce − 0,7±1,0	e 1,6±2,0	e − 1,9±1,7	abcd : p 0,001 f : p 0,005 e : p 0,01
pCO_2 (mm Hg)	a 45,2±5,1	abc 35,6±2,7	b 44,9±3,1	cd 43,0±1,5	 42,4±1,9	d 39,9±3,0	abc : p 0,001 d : p 0,05

Values with the same superscript differ significantly from one another — the level of this significance is presented in the last column.

Plasma levels of creatine kinase (C.P.K.), lactate dehydrogenase (L.D.H.), sodium and potassium. The forced exercise caused a definite elevation in the C.P.K. and potassium values but had no effect on the sodium concentration (Figs. 2 & 4 and Tables 1 & 2). The C.P.K. values reached a peak level after 2 h. The mean value for the treated group was higher than the mean for the untreated groups, but this was not statistically significant. There was an overall rise in the L.D.H. level, which, however, did not reach statistical significance except when the treated group at 2 h is compared with the stress samples (Fig. 3 and Table 1).

Dreyfus, Schapira & Schapira (1958)[2] have shown that A.C.T.H. and cortisone treatment causes hyperaldolasaemia and the elevation of several enzymes in the serum. This is possibly the reason for the difference between C.P.K. and L.D.H. levels of the treated and untreated groups, as treatment included the administration of glucocorticoids.

There was also a rise in the C.P.K. and L.D.H. plasma levels after stress in the springbok experiments[3]. In those experiments the enzymes reached much higher levels and took much longer to return to normal. When one compares the results of the different experiments it becomes clear that C.P.K. is a more sensitive index of forced muscle exercise. The L.D.H. takes longer to reach a peak and it would appear that the damage must be greater before L.D.H. starts to leak out.

Exercise stress caused a significant increase in plasma levels of potassium, whereas the subsequent treatment resulted in a highly significant reduction in potassium which persisted to 5 h. The rise in potassium after stress can be associated with the metabolic acidosis and increased cellular permeability. Grob (1957)[4] suggested that the release of potassium during muscular exercise might be the result of the dephosphorylaling action of creatine phosphate and adenosine triphosphate, as well as of glycolysis. Another possible explanation for the increased efflux of potassium ions from the cells to the plasma, is the fact that it has been shown that when the hydrogen ion concentration of extracellular fluid is increased an exchange occurs between the extracullular H+ and the intracellular K+[1]. This may also explain why the treated group exhibited a low plasma potassium as in this group hydrogen ion concentration must have been effectively buffered by the massive dosage of sodium bicarbonate.

It is possible that the body controls the sodium concentration more effectively, because the treated group also showed no significant difference from the untreated group, despite the fact that the treated sheep received 1 litre, 1,4% sodium bicarbonate.

pH, standard bicarbonate, pCO_2, buffer base and base excess. The mean pH immediately after exercise was significantly lower than the control value. Two hours later the untreated group had returned to normal, while the treated group had a significantly higher pH which persisted for 5 h (Table 2). The buffer base, standard bicarbonate and base excess showed exactly the same pattern (Table 2). Forced exercise lowered the standard bicarbonate values

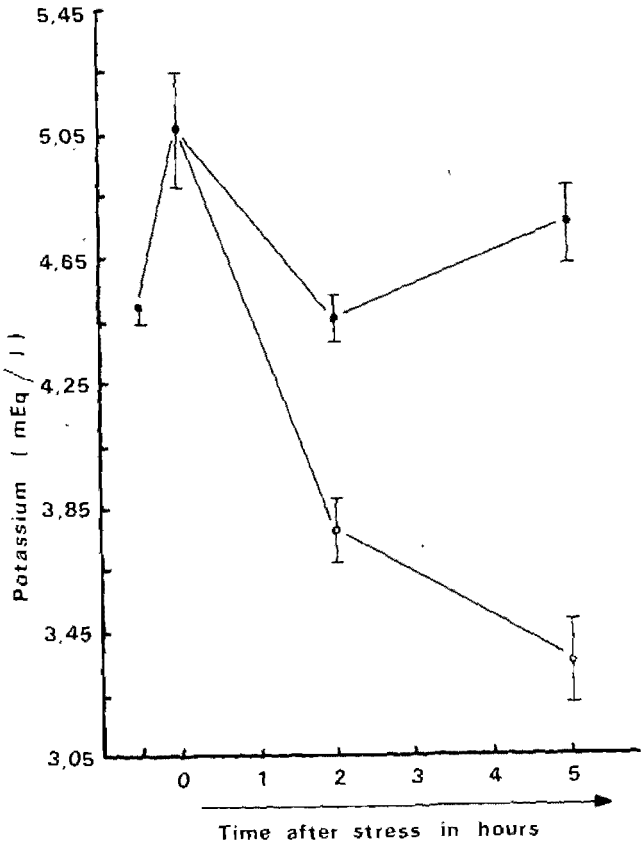

Fig. 4: The effect of forced exercise and subsequent treatment on the plasma levels of potassium
○ Treated group (± S.E.)
● Untreated group (± S.E.)

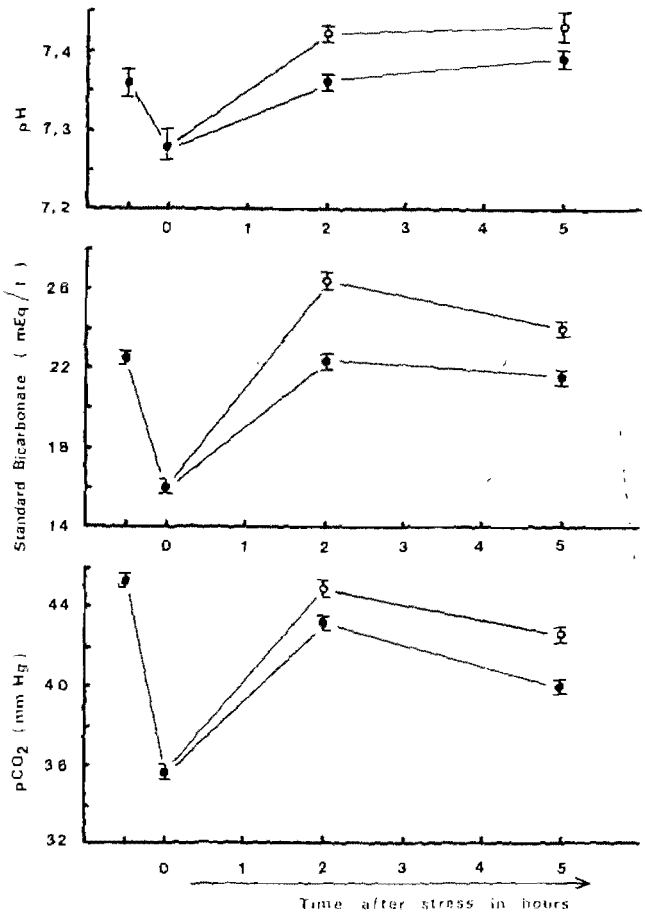

Fig. 5: The effect of forced exercise and subsequent treatment on blood pH, standard bicarbonate and pCO_2
○ Treated group (± S.E.)
● Untreated group (± S.E.)

significantly. Again the untreated group exhibited a normal standard bicarbonate after two hours while the treated group showed a significantly higher value, which again persisted to 5 h. In both the treated and untreated groups the reduced pCO_2 value after exercise returned to normal within two hours (Table 2).

The pH of blood represents a balance between volatile acids (respiratory component) and non-volatile acids or bases (non-respiratory component). During exercise metabolic acids are formed which cause a metabolic acidosis. The buffering system of the blood attempts to neutralize these acids with a resultant fall in the standard bicarbonate level (buffer base and base excess), which can be seen in the values of samples taken directly after stress. The respiratory system also responds (hyperventilation) to modify pH in the direction of normality.

The hyperventilation during forced exercise was sufficiently intense to result in a respiratory alkalosis, as can be seen in the low pCO_2 values directly after exercise. However, as Fig. 5 indicates, buffers and respiratory control could not fully compensate for the metabolic acidosis caused by exercise, with a resultant fall in the pH. The lowered pH, standard bicarbonate and pCO_2 therefore represents a partially compensated metabolic acidosis.

The higher pH of the treated group can be ascribed to the high sodium bicarbonate dosage in the treatment. As the pCO_2 was normal this increased pH reflects an uncompensated alkalosis in these animals at 2 and 5 h.

The values of the untreated group show that the stress was not very intense because the animals were able to restore a normal acid-base balance spontaneously within two hours.

Harthoorn and van der Walt (1973)[5] also found a reduction in the blood pH of blesbok that had been subjected to forced exercise. The lowest pH values were exhibited by the animals that had run the shortest distance and the highest pH values by those that had run the longest distance. After exercise the plasma bicarbonate, base excess and pCO_2 were also low with a slow return to normal within a few hours. This corresponds closely to the results obtained in the present experiment on sheep. Unfortunately the one blesbok that exhibited an alkaline pH after exercise cannot be compared with our results because it is not clear to what extent metabolic and respiratory components contributed to its alkaline pH.

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BOOK REWIEW

RESENSIE

STUDIES IN BIOLOGY NO.53
BONE AND BIOMINERALIZATION

K. SIMKISS

Edward Arnold (Ltd), London, 1975
pp IV Figs 31 Publ. Price £1.15 sterling

The problem of the exact nature of biomineralization has existed for many years. Although all problems have not yet been solved, the author has succeeded in discussing certain key experiments which have led to some of the present day concepts on the physiology of bone. He has gone into the role of the theory of epitaxy, the action and nature of certain crystal poisons and body fluids, as well as the role of the parathyroid mormone and of calcitonin in mineral deposition or resorbtion. This booklet has not been written as a textbook but merely as a presentation of problems with experiments attempting their solution. For the benefit of the hasty reader the final chapter comprises a very neat summary of the present knowledge of biomineralization with references for further reading.

W.H.G.

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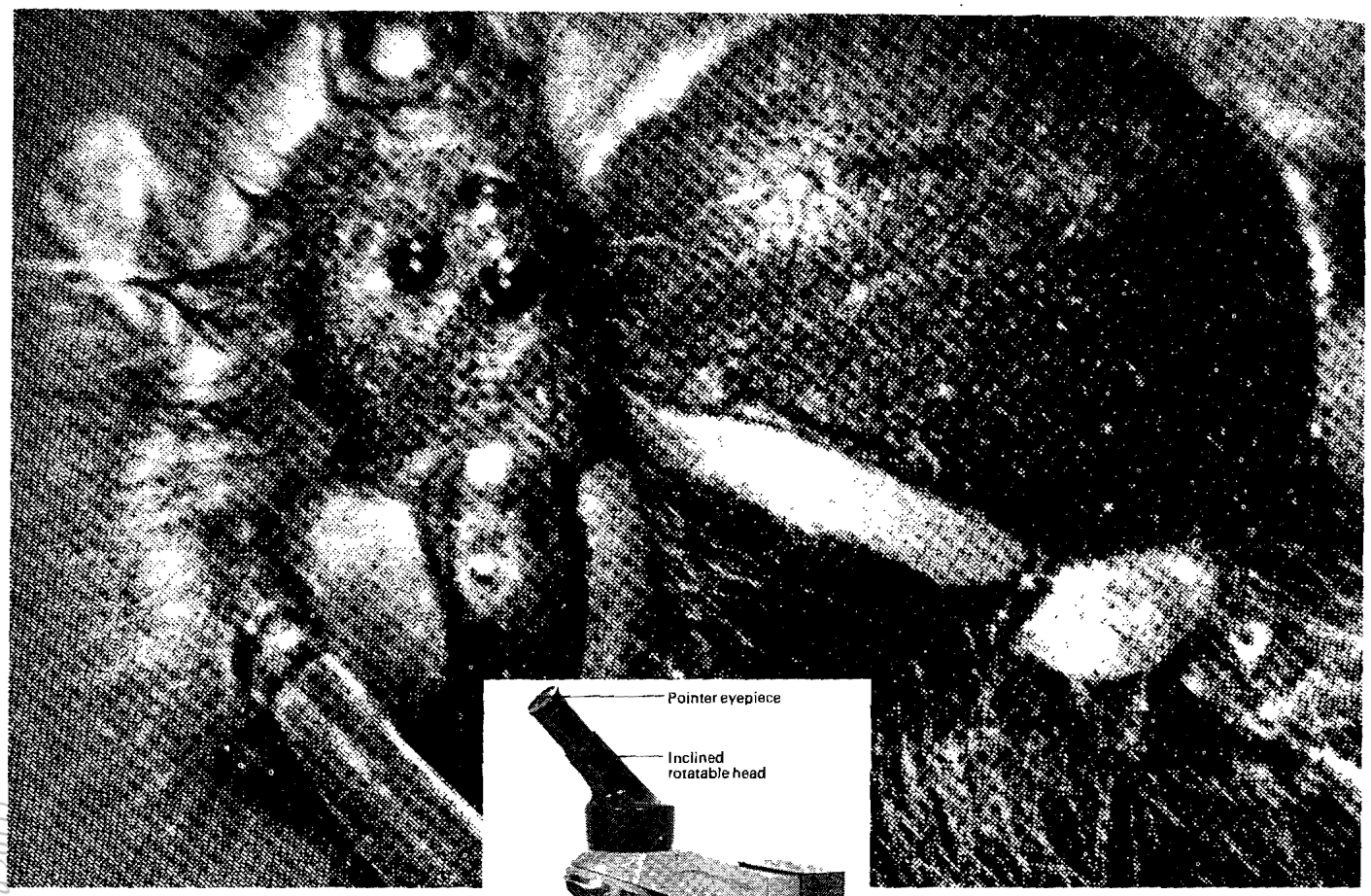

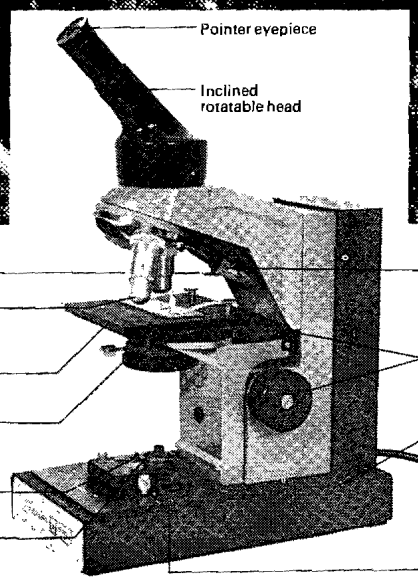

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THE CAPTURE AND TRANSLOCATION OF GEMSBOK ORYX GAZELLA GAZELLA IN THE NAMBI DESERT WITH THE AID OF FENTANYL, ETORPHINE AND TRANQUILLIZERS

H.EBEDES*

SUMMARY

Twenty-three gemsbok in the Namib Desert were captured with combinations of fentanyl or etorphine hydrochloride, hyoscine hydrobromide and tranquilizers such as azaperone, SU – 9064, triflupromazine hydrochloride and acetylpromazine maleate. Fentanyl, a New imbolizing compound, proved to be a safe and effective immobilizing drug for capturing gemsbok.

The gemsbok were chased on the interdune plains and darted from a Land Rover with the Palmer powder-charge Cap-Chur gun. A six-seater helicopter was used on a trial basis to dart a gemsbok, but it is suggested that a smaller, more manoeuvrable helicopter be used for futher operations.

All the gemsbok were transported under narcosis from the capture area to an enclosure.

Chlorpromazine hydrochloride was injected into the captured gemsbok to sedate them in their new confined environment. Tranquillizers such as chlorpromazine hydrochloride, acetylpromazine maleate and a new tranquillizer SU – 9064 were used to sedate the animals during long distance transportation in crates. This prevented the animals from injuring themselves and damaging the crates.

For the first time in South West Africa wild animals were transported by air. A journey by road which under normal circumstances would have taken over 40 hours, was completed in less than 9 hours by utilizing aerial transportation. There were no losses during transportation and only two gemsbok were injured during the translocation operation.

INTRODUCTION

During April and May 1968 several farmers in the arid areas bordering the restricted Diamond Area 2, south-east of Walvis Bay and west of the Naukluft Mountains, reported and complained that hundreds of gemsbok *Oryx gazella gazella* had moved onto their farms and were competing for grazing with their domestic stock. These reports were confirmed by the South African Police who regularly flew helicopter patrols along the borders of the diamond areas. Prior to farming activities in this marginal area, herds of gemsbok normally migrated eastward from the dune area to the escarpment. This was particularly the case in times of drought. The erection of fences and other farming activities restricted this migration. During the migrations hunting accounted for the loss of many animals over the years.

A small number of gemsbok from South West Africa had been promised to the Transvaal Nature Conservation Department to revitalise their existing small gemsbok herd on Langjan Game Reserve in the Northern Transvaal, South Africa. Because surveys had shown that large numbers of gemsbok were present on the farm "Elim" and the capture operation took place there. The area was free from foot-and-mouth disease and the captured animals could be moved from the farm to Windhoek without lengthy quarantine measures.

Attempts in the past to capture gemsbok with nets were unsuccessful and high mortality resulted from the animals fracturing their necks in the nets or from animals which had to be destroyed because of fractured limbs and other injuries. Driving the animals into specially constructed bomas could not be considered for two reasons; firstly, personnel and motor vehicles required for such an operation were not available, and secondly, gemsbok frequently gore each other fatally when confined or cornered in a small area especially after being chased or subjected to human interference.

Gemsbok have been successfully captured with the aid of etorphine hydrochloride (M-99, Reckitt) and tranquillizers[1]. Trial quantities of a new neuroleptic drug fentanyl (Janssen Pharmaceutica) and a butyrophenone derivative azaperone (Janssen Pharmaceutica) were received from the South African distributors, Messrs. Ethnor (Pty) Ltd., P.O. Box 1934, Johannesburg. The capture-project presented an opportunity to test the efficacy of these drugs on gemsbok in the Namib.

This paper records the method used and the results obtained in capturing gemsbok in the Namib with fentanyl and/or etorphine in combination with hyoscine hydrobromide and various tranquillizers and transportation of these animals with the aid of tranquillizers from the Namib to Daan Viljoen Game Reserve and from Daan Viljoen Game Reserve near Windhoek, S.W.A. by air to the Langjan Game Reserve near Pietersburg in the Transvaal, South Africa.

MATERIALS AND METHODS

The Palmer powder-charge Projector and standard 2 ml, 3 ml or 5 ml Palmer darts with barbed needles were used through-out for darting the gemsbok.

The following immobilizing and tranquillizing drugs and morphine-antagonists were used:

Etorphine hydrochloride (M-99 Reckitt).

The use of this synthetic morphine derivative for capturing gemsbok specifically has been described by Ebedes[2] and for capturing a wide variety of wild animals by several workers[1 3 8 10 12 14 16]. At present

Present Address: Private Bag X5020 Stellenbosch, 7600.

etorphine is the most extensively used chemical compound for capturing wild animals. A solution of 10 mg/ml etorphine was prepared by dissolving the powder in the tranquillizers – either Siquil (Squibbs) or Acetylpromazine (Boots).

Fentanyl (Janssen Pharmaceutica).

Fentanyl is a short-acting narcotic analgesic similar in action to etorphine, but 10 times less potent. Its use for immobilizing wild animals has been described in two separate papers by Pienaar [12 13] and Keep & Keep [9]. One and a half grams and 3,14g of fentanyl citrate powder was dissolved in 50 ml dimethylsulphoxide to make concentrations of 20 mg/ml and 40 mg/ml respectively as described by Pienaar [13].

Azaperone (Janssen Pharmaceutica).

Azaperone is a new neuroleptic drug used in combination with fentanyl. Azaperone produces rapid and consistent sedation with a wide margin of safety, and without deleterious effects on body temperature and cardiovascular activity. Solutions containing 100 mg/ml were prepared by dissolving 10 grams azaperone base and 4,8 grams tartaric acid in 20 ml water and warming to 80°C. After the salts had dissolved the solution was made up to 100 ml and filtered.

Acepromazine maleate (Acetylpromazine, Boots Pure Drug Co.)

Triflupromazine hydrochloride (Siquil, Squibb Laboratory) and chlorpromazine hydrochloride (Largactil, May and Baker (Pty) Ltd.). These are well-known tranquillizers which have been extensively used and well-described in the literature.

SU – 9064 (Ciba Ltd.)

SU – 9064 is a new tranquillizer resembling reserpine. Trial samples of the 1% injectable solution were supplied by Messrs A.S. Ruffel (Pty) Ltd., Windhoek. SU – 9064 was used for sedating gemsbok during transportation.

Hyoscine hydrobromide (Burroughs Wellcome).

Hyoscine hydrobromide has an atropine-like action in that it depresses the central nervous system, reduces salivation and through its mydriatic effect causes temporary blindness. Solutions containing 100 mg/ml were prepared.

Morphine antagonists.

Nalorphine hydrobromide (Lethidrone, Burroughs Wellcome) and cyprenorphine hydrochloride (M-285, Reckitt). Both morphine antagonists were used to reverse the narcotic effect of fentanyl and etorphine. Nalorphine is normally used at a dosage rate of 25 mg per 1 mg etorphine and is available commercially in multidose solutions of 20 mg/ml. Cyprenorphine is used at 2,5 times the dosage rate of etorphine. The powder supplied by Messrs Reckitt and Colman (Pty) Ltd. of Cape Town was dissolved in sterile water to prepare a solution of 10 mg/ml. Cyprenorphine, which is the specific antagonist for etorphine was also used to reverse the effects of fentanyl.

Capture producedures.

The large interdune plain on the farm "Elim" contained up to 600 grazing gemsbok. A stripped-down open short-wheel base Land Rover fitted with roll-bars was used to chase a previously selected group of animals. When a suitable animal was seen it was driven away from the group and darted from a distance of 10 to 25 m at speeds which varied from 32 to 56 km per hour. If an animal could not be darted within 1 minute from the start of the chase it was left alone and another group selected. This precaution was to prevent stress and overexhaustion which could result in muscular dystrophy. After an animal was darted, it was left to run until it became affected by the drugs. On occasions an animal would attempt to escape by running into the sand-dunes and it was prevented from doing this by driving between the fleeing animal and the dune. Capturing on hot days was undertaken only during the early hours of the morning from sunrise to approximately 11h00, so that hyperthermia could be avoided.

After the gemsbok were recumbent, short lengths of rubber hose pipe were fitted over each horn, the eyes were blindfolded and the rectal temperature, heart rate and respiratory rate were taken and recorded. Antibiotics such as Penicillin ("Aquacillin", A.S. Ruffel; "Hostacillin", Hoechst Pharmaceuticals); Tetracycline ("Safmycin", A.S. Ruffel); Corticosteroids ("Vercortenol", Ciba; "Delta-cortril", Pfizer) and vitamins ("BO-SE", H.C. Burns or "Vit. A.D.E.", Pfizer) were routinely injected intramuscularly into each animal to counteract infection and stress which may have resulted from the capture procedure.

Each animal was injected intramuscularly with 50 to 100 mg chlorpromazine hydrochloride, loaded onto the back of the Land Rover and transported sometimes up to 20 km under anaesthesia to a large circular communal enclosure which was constructed from 2,45 m high diamond-mesh netting wire covered on both sides with hessian. At the enclosure a number of gemsbok were weighed while under anaesthesia. The morphine antagonist was then injected intravenously and the recovery time recorded.

A six-seater South African Police Allouette helicopter was used on a trial basis to dart one gemsbok. The procedure was unsuccessful because the helicopter was found to be too fast and could not be manoeuvred for darting the fast-moving gemsbok.

The animals were fed veld grass collected daily in the surrounding area and also baled dry lucerne hay and Epol Antelope cubes (a commercially produced concentrate feed).

When the required number of gemsbok had been captured, each one was recaptured in the enclosure by lassoing with a rope, injected intramuscularly with either chlorpromazine hydrochloride (0,44 to 0,66 mg/kg) or SU – 9064 (0,11 to 0,13 mg/kg) and loaded into wooden crates for transprtation to Daan Viljoen Game Reserve, near Windhoek. The floors of the crates were covered with 4 to 5 cm of sand. Because of the high environmental temperatures during the day, the animals were loaded during the late afternoon and transported during the night. The distance travelled was just over 500 km and on arrival were released into small enclosures for an acclimatisation period. During this period they were fed lucerne hay and Epol Antelope cubes.

After the acclimatization period of 2 months the gemsbok were lassoed, injected with an average dose of 1,4 mg acepromazine maleate and loaded into

crates by officials of the Transvaal Nature Conservation Capture Unit. They were then transported by trucks to the Eros Airport near Windhoek and loaded into a South African Airforce Hercules Transport aieroplane and flown to Pietersburg in the Northern Transvaal. From Pietersburg they were transported by truck to the Lanjan Game Reserve, a distance of about 125 km, and released after 4 weeks of acclimatisation.

RESULTS

A total of 23 gemsbok was captured in 7 days. The results of the immobilization of 18 gemsbok are presented in Tables 1 and 2. The immobilizing data of five gemsbok captured are not recorded for the following reasons: One animal was destroyed because the right tibia was fractured by the impact of a dart. Two gemsbok accidentally darted in the abdomen and one accidentally darted in the thorax were destroyed because the darts could not be retrieved without major surgical intervention and the animals were suffering. The fifth gemsbok was found to be blind in one eye and was released immediately after capture because it was unsuitable for the Langjan Game Reserve.

Four of the twelve gemsbok darted with fentanyl had to be darted a second time because they did not show signs of becoming immobilized sufficiently after

Table 1: CAPTURE OF NAMIB GEMSBOK WITH FENTANYL (JANSSEN)

No.	SEX	WEIGHT kg	DART SITE	FENTANYL mg	Hyoscine mg.	Azaperone mg.	Acetylpromazine mg	IMMOBILIZATION TIME	Temperature °F	Pulse per min.	Respiration per min.	NALORPHINE mg	RECOVERY TIME
1	F	c 160	Jaw	40	10	80	–	19 min	107.4	64	26	100 I.V.	30 sec.
2	F	c 180	Neck	40	10	80	–	12 min 43 sec.	107.2	54	24	80 I.V.	16 sec.
3	F	95	Abdomen	40	10	80	–	12 min 38 sec.	106.4	–	–	100 I.M.	2 min 30 sec.
4	F	195	Ear	40	10	80	–						
			Hip	40	10	80	–	11min 30 sec.	104.2	76	58	80 I.M.	2 min 43 sec.
5	F	180	Hip	40	20	80	–						
			Shoulder	40	–	80	–	37 min 30 sec.	107.6	80	62	80 I.M.	2 min 45 sec.
6	F	117	Scapula	60	30	–	–						
			Hip	20	–	–	5	36 min	108.5	84	56	50 I.V.	1 min 20 sec.
7	F	98	Hip	60	30	–	5	13 min	107.4	68	48	50 I.V.	42 sec.
8	F	109	Hip	60	30	–	–						
				12	–	–	5	14 min	105	60	48	60 I.V.	28 sec.
9	F	70	Shoulder	60	30	–	15	8 min 28 sec.	105.3	84	90	50 I.V.	Overdose of
												+20 I.M.	Fentanyl.
												+40 I.M.	Recovered in
												+20 I.M.	9 min 20 sec.
10	F	87	Hip	30	20	–	15	12 min 46 sec.	106	80	62	40 I.V.	17 sec.
11	F	82	Hip	30	20	–	15	9 min 20 sec.	106.8	84	64	20 I.V.	10 sec.**
12	F	204	Shoulder	60	20	–	20	9 min 35 sec.	106.2	68	60	M285 12 mg I.V.	38 sec.

c = estimated body weight

**= Gemsbok started to recover before Nalorphine was injected, hence rapid recovery-time

Table 2: CAPTURE OF NAMIB GEMSBOK WITH ETORPHINE HYDROCHLORIDE (RECKITT)

NO.	SEX	WEIGHT kg	DART	ETORPHINE mg	Hyoscine mg	Siquil mg	Acetylpromazine mg	IMMOBILIZATION TIME	Temperature °F	Pulse per min	Respiration per min	Cyprenorphine mg	RECOVERY TIME
1	F	c 136	Neck	2,1	10	30	–	20 min	104,0	70	42	5	1 min 22 sec.
2	F	c 200	Withers	3	15	40	–	3 min 30 sec.	104,2	66	36	6	8 min 30 sec.
3	M	93	Shoulder	2	15	40	–	12 min 8 sec.	105,5	68	40	4	56 sec.
4	F	82	Hip	3	15	40	–	8 min 35 sec.	105,0	–	–	4	45 sec.
5	M	c 250	Hip	3	20	–	20	28 min	105,4	68	48	6	1 min 10 sec.
6	F	113	Neck	2	15	–	10	3 min 20 sec.	105,0	64	28	4	1 min 5 sec.

c = estimated body weight.

10 minutes and would have escaped into the sand-dunes.

The three tranquillizers, chlorpromazine hydrochloride, acepromazine maleate and SU – 9064 were ideal for sedating the gemsbok during transportation. Most of the animals showed signs of sedation within 30 minutes of injection and lay in sternal recunbency for most of the journey. Only two accidents occured during transportation to the Langjan Game Reserve. One gemsbok fractured a horn and a second fractured a leg which was set in plaster by a veterinarian.

DISCUSSION

The capture and translocation of gemsbok were successful and contained several procedures which had not previously been attempted or recorded in wildlife capture operations in South West Africa.

The injection of a tranquillizer to calm the captive animals prevented fighting among the newly caught animals and helped them to adjust to their new confined and strange surroundings. In previous capture and holding operations several gemsbok were lost through fatal wounds inflicted while fighting. Two methods are usually used to prevent goring and these are to clip the sharp tips of the horns or to cover the tips of the horns with tightly-fitting rubber hose pipes. This last method is not recommended if the animals are to be confined for a long period because if the pipes are secured firmly with wire the horns often become gangrenous particularly in young animals.

Transporting the animals under narcosis from the capture area to the holding pen was so successful that one can speculate on the possibility of transliocating animals over longer distances in the future and thus eliminating the use of crates. In Etosha National Park two gemsbok were transported under narcosis for more than 60 kilometers on the back of a Land Rover without any signs of stress or discomfort.

When aggressive animals such as gemsbok, are transported in crates, injuries are frequent and the crates are often damaged. By using suitable tranquillizers for sedation the percentage of injuries is limited, the animals are calm during the journey and arrive at their destination in a less stressed condition.

Although black rhinoceros *Diceros bicornis* have been captured successfully in S.W.A. with the aid of a helicopter, this was the first time a helicopter was used for darting gemsbok. A smaller, more manoeuvrable helicopter such as the Bell two-seater would be preferable to the large Allouette. Using a helicopter a large number of animals could be captured in a short period. The helicipter could be of great assistance in turning the fleeing gemsbok away from the sand-dunes which are virtually inaccessible to even four-wheel drive vehicles. The possibility also exists that the helicopter could be used to airlift animals out of the sand-dunes in a net attached to its undercarriage.

It is estimated that the journey by road along the shortest route from Daan Viljoen Game Reserve to Langjan Game Reserve would have taken more than 40 hours. The time taken from the crating of the first gemsbok in Daan Viljoen Game Reserve to the offloading of the last gemsbok in Langjan Game Reserve was less than 9 hours. More than 30 hours of travelling time was thus saved by using air-transport.

Fentanyl in combination with hyoscine hydrobromide and azaperone or acetylpromazine proved to be a suitable immobilizing drug for capturing gemsbok. Insufficient data is available for a statistical comparison between the effects of fentanyl and etorphine and a comparison of the two immobilizing compounds was not the purpose of this paper.

ACKNOWLEDGEMENTS

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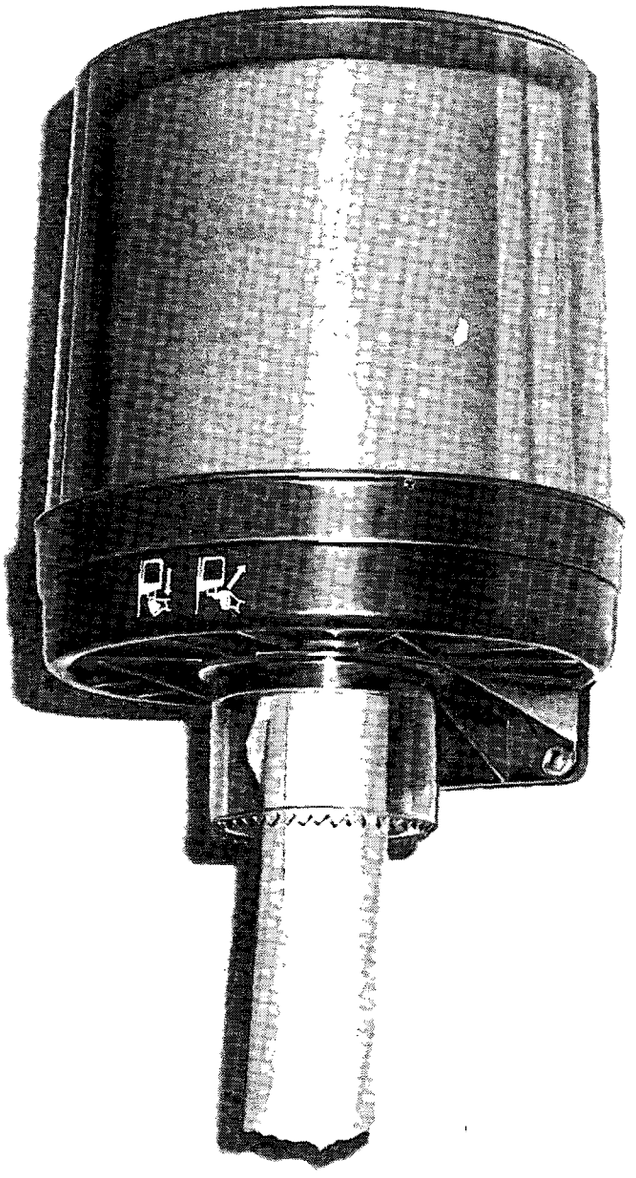

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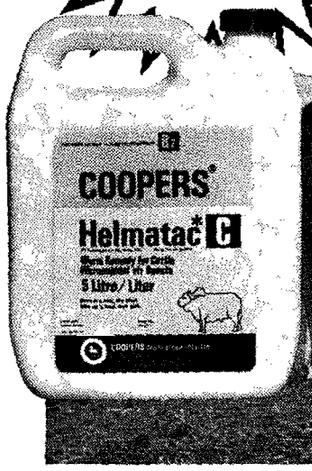

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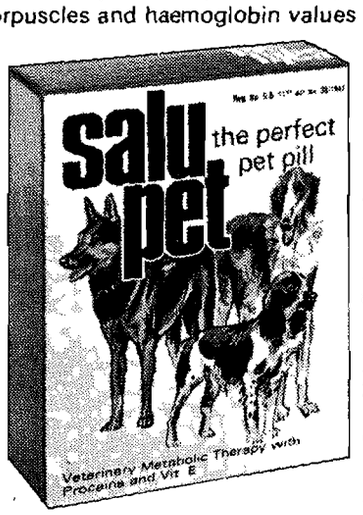

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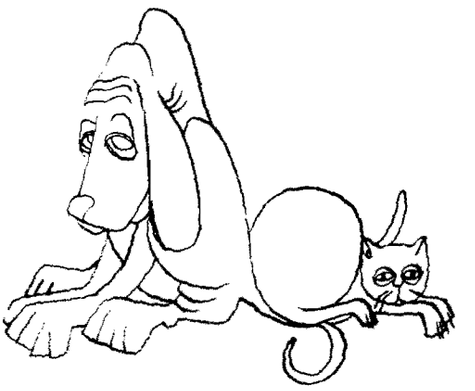

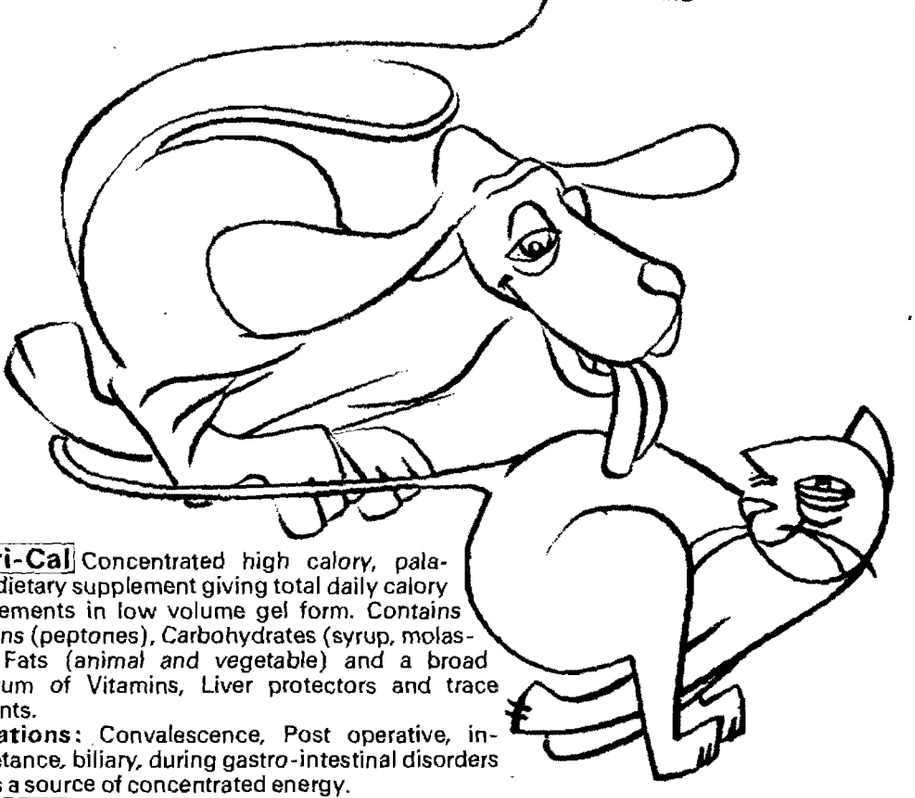

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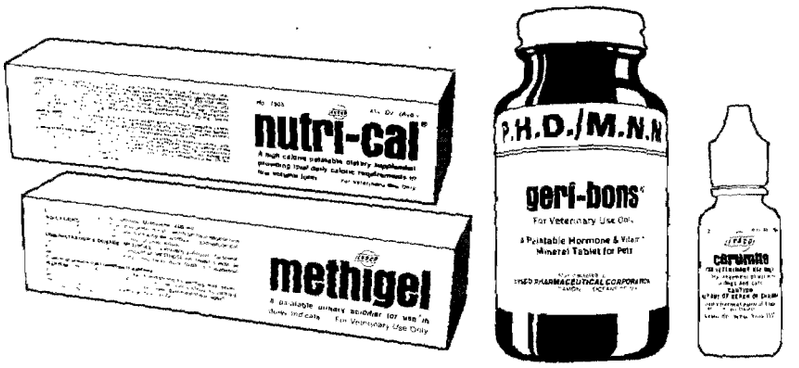

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SERUM-GRONADOTROFIENAKTIWITEIT VAN DRAGTIGE SEBRA- EN PERDEMERRIES

J.F.W. GROSSKOPF* EN G.L. SMUTS**

SUMMARY

Blood was collected from 28 zebra mares *(Equus burchellia antiquorum)* immediately after being shot in the Kruger National Park. The serum was separated within two hours after collection and then stored at -15°C for later assay. Of these, thirteen selected samples were tested for gonadotrophic activity. The stage of pregnancy was determined from a foetal growth curve. Blood samples from pregnant horse mares were collected by venipuncture. Nine mares were sampled. Seven blood samples at different stages of pregnancy were collected from one mare, four from another and only one sample each from the other seven mares. The stage of pregnancy was calculated from the date of last service.

The levels of gonadotrophic activity of the serum samples were estimated through its effect on the weight of mouse ovaries. Five groups of five virgin female white mice were injected subcutaneously with zero, 0,025 ml, 0,075 ml and 0,1 ml of serum per mouse on two successive days and killed by ether inhalation 48 hours after the last injection. The mean weight per overy was determined for each level of every sample injected and a dose: response curve drawn from which the percentage increase in ovarian weight caused by the 2 x 0,05 ml dosage level of each serum sample was estimated.

The curves obtained by plotting gonadotrophic activity at various stages of pregnancy for horse and zebra mares are generally similar but differ in certain details. PMS obtained from horses has a greater activity and appears to be secreted over a shorter period of time i.e. it disappeared by the 160th to 180th day of pregnancy. In zebra mares, on the other hand, a relatively lower activity was found during the peak period (65th to 80th days) but it was maintained loger and was still detectable at 229 days but absent at 365 days after conception.

INLEIDING

Die gonadotrofiese aktiwiteit van dragtige merrieserum wat oorspronklik deur Cole en Hart[2] vasgestel is, is ook later in die endometriumbekers van sebramerries *(Equus burchelli boehmi)* gevind[6]. Volgens grafieke[6] het die gonadotrofiese aktiwiteit in die endometriumbekers 'n hoogtepunt bereik tussen die 80ste en 100ste dag na konsepsie. Vir die doel van 'n studie oor die groei, voortplanting en bevolkingsdinamika van sebras in die Nasionale Krugerwildtuin is 'n aantal sebramerries *(Equus burchelli antiquorum)* geskiet en van die geleentheid is gebruik gemaak om ook bloedmonsters te kollekteer vir hormoonstudies.

MATERIAAL EN METODE

Bloedmonsters is net na dood opgevang van 28 sebramerries in verskillende stadiums van dragtigheid wat in die Nasionale Krugerwildtuin geskiet is. Die serum is van die res van die bloed geskei en daarna by -15°C gestoor vir latere hormoonbepalings. Die stadium van dragtigheid van die onderskeie merries is deur middel van 'n fetale groeikurwe [8] bepaal. Die bloedmonsters van die perdemerries is gebloei uit die Vena jugularis van nege merries. Sewe monsters is op verskillende dae van een van die merries getap, vier van 'n ander merrie en net een monster van elk van die oorblywende sewe merries. Die bloed is toegelaat om te stol, daarna oornag by 5C gehou en die volgende dag is die serum afgegooi en bevries. Die dragtigheidstadium van die merries is bereken vanaf hulle laaste dekdatums.

Vir die bepaling van die gonadotrofienaktiwiteit van die serum is witmuise daarmee ingespuit en die invloed daarvan op die eierstokgewigte bepaal [7]. Vir elke bepaling is 25 jong vroulike witmuise (21-23 dae oud) in groepe van 5 elk verdeel. Die muise is volgens gewig ingedeel sodat die groepe se gemiddelde gewigte dieselfde was. Vier van die groepe is op elke van twee agtereenvolgende dae onderhuids ingespuit met respektiewelik 0,025 ml, 0,05 ml, 0,075 ml en 0,1 ml per muis en 48 uur na die tweede inspuiting met eter doodgemaak. Die oorblywende kontrolegroep is nie behandel nie en is saam met die ander doodgemaak. Die eierstokke is sorgvuldig uitgedissekteer en dié van elke groep is gesamentlik in klein voorafgeweegde houers met digsluitende proppies geplaas en geweeg. Die gemiddelde gewig per eierstok van elke groep is bereken en grafies met die toegediende dosis van dragtige merrieserum vergelyk. Met die relatief lae dosisse van dragtige merrieserum kan 'n reguit lyn in hierdie gevalle verwag word[5]. Alhoewel alle punte nie noodwendig op die lyn geval het nie kon, veral met die vaste nulpunt, 'n redelike betroubare helling vir elke monster vasgestel word. Vanaf hierdie grafiek is die ooreenstemmende waarde van die eierstokgewig toe bepaal vir die tweemaal 0,05 ml serumdosis. Die aldus berekende eierstokgewigte vir elke serummonster is toe uitgedruk as 'n persentasie van die kontrole eierstokgewigte.

RESULTATE EN GEVOLGTREKKINGS

Die persentasie wat twee inspuitings van 0,05 ml van die onderskeie sebra- en perdeserummonsters die muiseierstokke in gewig laat toeneem het word in Fig. 1 volgens die dragtigheidstadium van die merries aangedui.

* Dept. Diereproduksie, Universiteit van Pretoria, Pretoria. 0002
** Afd. Natuurbewaring, Nasionale Parkeraad, Privaatsak X404 Skukuza, 1350

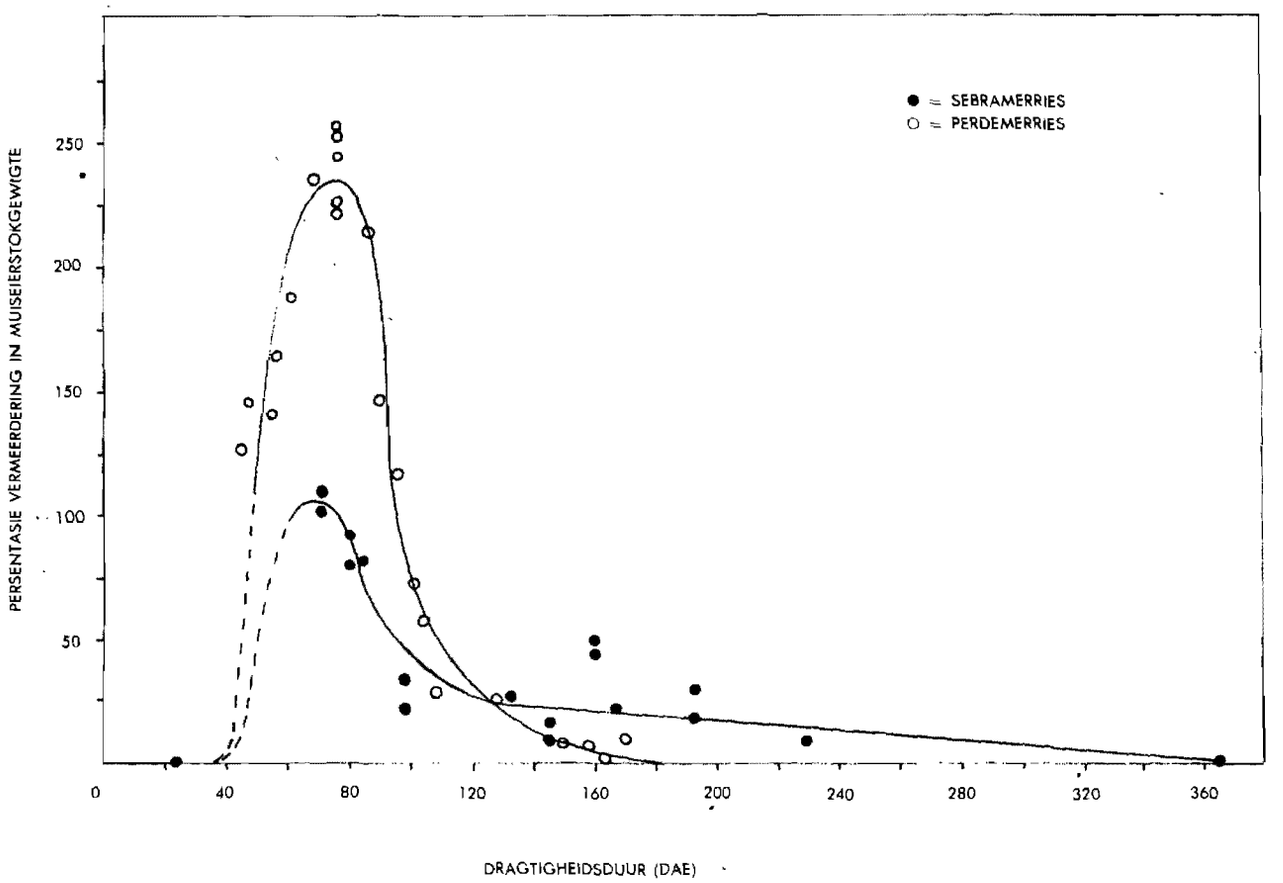

Fig. 1: 'n Vergelyking tussen die gonadotrofiese aktiwiteit van dragtige sebra- en perdemerrieserum. (Twee of meer punte op dieselfde dag verteenwoordig herhaalde bepalings op dieselfde monster).

Fig. 1 toon duidelik dat die serumgonadotrofien-aktiwiteit van beide die perde- en sebramerries vinnig styg na ongeveer die 40ste dag van dragtigheid en dat dit 'n piek bereik tussen die 65ste en 80ste dag na konsepsie. Soos gesien kan word was die maksimum gonadotrofieninhoud van perdemerrieserum aansienlik hoër as dié van die sebramerries. 'n Ander opvallende verskil is dat die gonadotrofiese aktiwiteit in die serum van perdemerries nie na die 180ste dag van dragtigheid vasgestel kon word nie terwyl bepaalbare hoeveelhede nog op die 229ste dag van dragtigheid in 'n sebramerrie se serum teenwoordig was.

Hierdie gevolgtrekkings ten opsigte van die perdemerries stem ooreen met vroeëre bevindings[1 3 4]. Die resultate van hierdie ondersoek ondersteun ook die bevindings dat die gonadotrofiene in die endometriumbekers van dragtige sebramerries nie dieselfde hoë peile bereik as by perdemerries nie[6] en dat dit verband mag hou met die relatief min sekondêre ovulasies wat in dragtige sebramerries voorkom[8].

DANKBETUIGING

Die Direkteur, Navorsingsinstituut vir Veerartsenykunde word bedank vir die beskikbaarstelling van die groot getalle witmuise wat vir hierdie ondersoek benodig was.

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THE RESISTANCE OF A FIELD STRAIN OF *HAEMONCHUS CONTORTUS* TO FIVE BENZIMIDAZOLE ANTHELMINTICS IN CURRENT USE

J. BERGER*

SUMMARY

Using artificially infected sheep, the response of a laboratory strain of *Haemonchus contortus*, which had not been subjected to anthelmintics for three years, was compared with that exhibited by a parbendazole resistant field strain following treatment with each of seven anthelmintics. the laboratory strain proved fully susceptable to thiabendazole, parbendazole, cambendazole, mebendazole, fenbandazole, levamisole and haloxon, in contrast to the field strain which showed a moderate to marked resistance to all five benzimidazoles, but was fully susceptible to levamisole and haloxon.

INTRODUCTION

The introduction of phenothiazine as an anthelmintic in 1939 and its rapid adoption for regular use by sheep farmers throughout the world was frequently followed by its continuous use during more than a decade. Indications that some nematodes were becoming resistant to medication on those premisses on which phenothiazine had been used for the longest periods were first recorded in 1953. Following up this field evidence, Drudge *et al*[6] showed that there was a marked resistance by strains of mixed nematodes, preponderantly *Haemonchus contortus*, to both full therapeutic and low daily doses of phenothiazine previously proven to be effective. In further trials *H. contortus* isolated from these field infections were found to exhibit a resistance threshold up to eight times that of other strains[7]. On the other hand, Sinclair[16] was unable to provoke any increased resistance to phenothiazine by the sixth generation of *Thrichostrongylus colubriformis* passaged and exposed to medication in the laboratory. This exposure however would not have simulated the selection pressure encountered by field strains in sheep dosed several times a year for a number of years.

In contrast to the slow build up of resistance to phenothiazine, reports of resistance by *H. contortus* were recorded in the United States within three years of the introduction of thiabendazole. Conway[4] experienced some variation in the efficacy of the drug since its first use in 1961 and it is possible that this was an example of strain tolerance withing the species. On the other hand field studies by Drudge *et al*[8] produced evidence of a rapid build up of resistance during the course of only three medications.

Similar reports of resistance followed from South America[15] and Australia[17]. Hotson *et al*[10] recorded field evidence of *T. colubriformis* resistance to thiabendazole as early as 1967.

Using known thiabendazole resistant strains of *H. contortus*, several authors have subsequently recorded cross resistance to pardendazole, [18] [11] as did Hotson *et al*[10] using strains of *T. colubriformis* resistant to thiabendazole.

More recently Kates *et al*[12] and Colglazier *et al*[3] have described the experimental development of a strain of *H. contortus* resistant to cambendazole.

Little has been published on the development of resistance to organo-phosphate anthelmintics and Douglas and Baker[5], who recorded two strains of *Ostertagia circumcincta* showing varying response to an unspecified organo-phosphate, had good reason to suggest that this was also an example of genetic strain tolerance within a species.

HISTORY

During January 1975 the presence of a heavy burden of *H. contortus* in the abomasum of a lamb, dosed with parbendazole four days previously, prompted a farmer from the Boshof district to report this apparent failure in efficacy. Using four different anthelmintics, Lloyd[13] undertook confirmatory dosing of marked lambs on these premises and showed that both parbendazole and fenbendazole failed to eliminate adult *H. contortus*, in contrast to a highly efficent result obtained with levamisole or haloxon.

Continuous short interval nematode control with parbendazole on these premises during the past six years had provided the anthelmintic exposure conducive to the development of resistance. Thiabendazole was not used prior to this, nor have this or other benzimidazoles been given more recently.

A donor sheep was infected with the resistant (designated Boshof) strain of *H. Contortus* and a pilot laboratory trial, using nine artificially infected sheep, confirmed resistance to parbendazole and full susceptibility to haloxon. The purpose of this report is to record the results obtained in 'n larger trial designed to obtain the maximum information about any cross resistance to the other benzimidazoles in current use, exhibited by adults of this field strain, while at the same time comparing anthelmintic efficiency against this and the normal laboratory strain of *H. contortus*.

MATERIALS AND METHODS

Experimental sheep

Eighty Merino lambs, eight to nine months old, were obtained from a farm on which regular anthelmintic treatments are given but on which they would have had some previous exposure to nematodes. The lambs were housed on slats before and during the trial, received a ration of lucerne chop and concentrates and were rendered worm-free by dosing with parbendazole and levamisole two and one week prior to infection respectively.

* Kwanyanga Research Station, Coopers (South Africa) (Pty) Limited, Greenfields, Republic of South Africa.

Experimental infections

Forty lambs in Groups 1 to 8 received infective larvae from a donor sheep carrying the normal (N) strain of *H. contortus* maintained over 12 passages in sheep at this laboratory over the last three years, during which time it had not been subjected to any anthelmintic.

Forty lambs in Groups 1A to 8A received larvae from a donor sheep carrying the Boshof resistant (R) isolate.

Each lamb received by stomach tube on each of three consecutive days, 1000 infective larvae, harvested seven to nine days previously.

Treatment schedule

Faecal worm egg counts carried out the day before treatment were used as a guide to the re-allocation of a few lambs between groups earlier selected at random.

Seven groups of five lambs received anthelmintic medication and one group remained untreated in the 40 lambs infected with either of the two strains. Treatments were carried out by tubing into the upper oesophagus, to simulate the more common method of field dosing, 21 days after the second day of infection.

The five benzimidazoles and two other compounds used were from standard commercial packs and were given at the recommended dose rate, or that at which registration had been obtained, according to the following schedule:-

Groups 1 & 1A Parbendazole: 30 mg/kg (Helmatac. 9% m/v suspension. Coopers (South Africa) (Pty Ltd.)

Groups 2 & 2A Thiabendazole: 44 mg/kg (Thibenzole. 14.76% m/v suspension. MSD (Pty) Ltd)

Groups 3 & 3A Cambendazole: 20 mg/kg (Bonlam. 3.33% m/v Suspension. MSD (Pty) Ltd)

Groups 4 & 4A Mebendazole: 15 mg/kg (Multispec. 5% m/v suspension. Ethnor Laboratories (Pty) Ltd.)

Groups 5& 5A Fenbendazole: 5 mg/kg (Panacur. 2.5% m/v suspension. Hoechst Pharmaceuticals (Pty) Ltd.)

Groups 6 & 6A Levamisole hydrochloride: 7.5 mg/kg (Nilwirm. 2.5% m/v solution. Coopers (South Africa) (Pty) Ltd.)

Groups 7 & 7A Haloxon: 20 mg/kg (Loxon. 8.38% m/v suspension. Coopers (South Africa) (Pty) Ltd.)

Observations

Faecal worm egg counts were only carried out prior to dosing with larvae to confirm freedom from infection and subsequently on the day before treatment and on the day of slaughter.

Lambs were autopsied six to ten days after treatment and the abomasum and proximal three metres of small intestine were processed to recover all mature *H. contortus*. As it was not possible to slaughter all treated and control lambs on one day, as suggested by Turton and Clark[19], recoveries were made from one lamb in each group on a single day to reduce the error associated with natural worm loss.

Samples of the largest male and female worms of both strains from untreated controls were held refrigerated in normal saline for physical examination and measurement. A comparison of heat-killed infective larvae was also made.

RESULTS

The results are summarised in the Table.

THE RESPONSE OF TWO STRAINS OF HAEMONCHUS CONTORTUS IN ARTIFICIALLY INFECTED LAMBS TO TREATMENT WITH SEVEN DIFFERENT ANTHELMINTICS

Groups of 5 lambs	Treatment and Dose Rate	N strain infection Groups 1 to 8				R strain infection Groups 1 A to 8 A			
		Mean eggs per gram of faeces		Mean no. of worms recovered (range)	Mean % reduction in worms burden	Mean eggs per gram of faeces		Mean no. of worms recovered (range)	Mean % reduction in worm burden
		on day before treatment	on day of slaughter			on day before treatment	on day of slaughter		
1 & 1A	Parbendazole 30 mg/kg	510	0	0	100	1 060	640	359 (207–642)	79.4
2 & 2A	Thiabendazole 44 mg/kg	650	0	2 (0–8)	99.9	1 430	1 825	1 085 (72–1530)	37.5
3 & 3A	Cambendazole 20 mg/kg	620	0	< 1 (0–3)	>99.9	1 170	730	584 (198–837)	66.4
4 & 4A	Mebendazole 15 mg/kg	630	0	0	100	1 180	1 020	642 (205–1125)	63.0
5 & 5A	Fenbendazole 5 mg/kg	520	0	0	100	1 490	200	334 (152–690)	80.8
6 & 6A	Levamisole 7.5 mg/kg	590	0	< 1 (0–1)	>99.9	900	0	0	100
7 & 7A	Haloxon 20 mg/kg	780	0	0	100	1 110	0	< 1 (0–3)	>99.9
8 & 8A	Untreated Controls	750	3 330	1 636 (423–2 152)	–	1 150	1 975	1 736 (1 339–2 148)	–

Worm recovery from the controls showed a satisfactory meand take of 54 and 57 per cent for the N and R strains respectively. The wider range in the N group may have been due to individual variation in immunity.

The near complete eradication of worms in all treated groups of lambs infected with the N strain of *H. contortus* conformed to the pattern expected after therapy with these proven compounds. In the groups infected with the R strain the decreased efficiency of the five benzimidazoles contrasted with an apparently unimpaired efficacy of levamisole and haloxon. Each benzimidazole had nevertheless exhibited considerable activity giving rise to mean worm reductions varying between 38 and 81 per cent.

Microscopic examination of worms of both strains, based on body measurements and scrutiny of the male and female genitalia, did not reveal any differences. Infective third stage larvae of each strain appeared identical although the mean total length of R strain larvae was 6 per cent greater than that of the N strain larvae.

DISCUSSION

The limitations set by the number of experimental sheep available did not allow for an assessment of response of both adult and larval stages of the R Strain to the benzimidazoles at increasing dose rates. Both Kates and Colglazier and their co-workers[11 2] using sheep with natural infections of *H. contortus* resistant to parbendazole, found that the larval stages were less susceptable than the adults. The same authors also recorded increasing efficiency at higher dose rates, although Theodorides *35 al*[18]. using a strain of *H. contortus* resistant to 50 mg/kg thiabendazole or 20 mg/kg parbendazole, showed that a threefold increase in dose rate resulted in a worm reduction of 94 and 99 per cent respectively, indicating that a small population of highly resistant worms had survived. The implications of the latter result must be considered when discussing with a stockowner the advisability of increasing the dose rate or changing to an unrelated anthelmintic.

The cross resistance to the four other benzimidazoles shown by the R strain in the present trial and its susceptibility to two other anthelmintics is in accordance with the findings of other workers. Smeal[17] quotes reports from Santos and Goncalves in Brazil that a benzimidazole-resistant strain of *H. contortus* was nontheless susceptible to trichlorphon and diiodonitrophenol.

Using a thiabendazole-resistant strain, Theodorides *et al*[18] found cross resistance to parbendazole, Kates *et al*[11] resistance to parbendazole but full susceptibility to levamisole, and Colglazier *et al*[1 2] resistance to parbendazole but full susceptibility to levamisole, pyrantel tartrate and rafoxanide. Using a thiabendazole-resistant strain of *Trichostrongylus colubriformis* Hotson *et al*[10] found cross resistance to parbendazole but full susceptibility to dl-tetramisole. The resistant strain of *H. contortus* used in the trial reported here proved not fully susceptible to fenbendazole, a finding at variance with that of Duwel[9] who found it effective against benzimidazole resistant strains of *Haemonchus* and *Trichostrongylus*.

Although the benzimidazoles may mainly exert their anthelmintic activity by inhibition of the nematode enzyme systems affecting energy production[14], the enzyme adaptation of strains which have become resistant has not increased their tolerance towards the cholinesterase inhibitory effect of organophosphates or the nerve ganglion stimulant effect of levamisole.

The results reported here confirm a reduced anthelmintic efficiency which had been patently obvious to a stockowner. At the same time they indicate that in spite of the range of worm reduction obtained following benzimidazole medication (38 to 81 per cent), not one of these compounds would, under the South African system of grading anthelmintic activity, obtain an A rating against adults of this R strain of *H. contortus* at the dose rates used. A search of the South African literature reveals little indication that benzimidazole anthelmintics are failing to substantiate the original claims of efficiency, but the local common necessity for adopting a regimen of frequent dosing must have exerted a selection pressure on populations of *H. contortus* as severe as those elsewhere. Evidence suggests that the continued use of these compounds on the majority of premises is still good practice, in the same way that the appearance of a resistant strain of tick species has not spelt the immediate withdrawal of an acaricide. Nevetheless increased efforts to investigate, confirm, record and advise on any suspected resistance should be made irrespective of the nature of the anthelmintic employed.

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BOOK REVIEW

RESENSIE

POISONOUS PLANTS OF AUSTRALIA

SELWYN L. EVERIST

Angus and Robertson, London, Sinhapore, Philippines and Australia, 1974. 64 Colour Plates, 64 Photographs, 42 Line Drawings, Publ. Price R51,00.

By virtue of the wide experience he has had through many years in the field of botany, the author is eminently suited to publish a book on poisonous plants in his country, including some common garden species. The book is divided into two sections.

Section 1 is subdivided into six chapters. Chapter 1 deals with the economic importance and history of plant poisoning. In Chapter 2 the methods of determining whether a plant is poisonous or not are discussed. Useful information on pitfalls which may be encountered, is supplied. In Chapter 3 the factors which play a role in determining the toxicity of plants are discussed. These factors concern the plant itself, the animal and the environment. Chapter 4 deals with methods of investigating plant poisoning in the field. Chapter 5 contains useful and interesting information on toxic substances in plants, i.e. metallic toxins, non-metallic mineral toxins and toxic organic compounds. Chapter 6 deals with copper, molybdenum and selenium poisoning as a result of their accumulation in pastures.

Section 2 is devoted to discussions on poisonous seed-bearing plants; ferns and fern allies; fungi, lichens, and algae.

The three appendices contain useful and important information on the distribution of poisonous plants, on poisonous plants grouped by symptoms, and on toxic principles in Australian plants.

All the illustrations are excellent. Referring to nitrate and nitrite poisoning in sheep and cattle, it is stated in the last line on page 271 that "The tongue and mucous membrances become bluish". This is not quite correct as the blood is chocolate-brown (methaemoglobinaemia) and consequently the visible mucous membranes are not bluish (cyanosis due to direct lack of oxygen in the blood) but leaden-grey in colour.

In discussing poisoning by *Solanum elaeagnifolium*, no mention is made of the publication by Buck, Dollahite and Allen (J. Amer. vet. med. Assoc. Vol 58, 1963, page 419). Apparently this *Solanum* species has caused considerable losses among cattle in Texas, USA. On page 555 the toxicity, symptoms and lesions induced by *Amanita muscaria* are discussed but no mention is made of the miosis which, among other symptoms, is a prominent feature.

It is stated that the book is intended for the use of graziers and farmers as well as professional advisers and it eminently fulfils this purpose. The book is a valuable addition to the library of all those interested in the toxicology of plants.

D.G.S.

TO THE EDITOR

AAN DIE REDAKSIE

CANINE HEARTWORM *(Dirofilaria immitis)*

Dear Sir:

Having lived in the U.S.A. for the past 3 years and having been active in clinical veterinary practice during this period, I feel it is my duty to bring to the notice of the responsible authorities and veterinary practioners, the potential threat posed to our canine population by importation of dogs infected with canine heartworm *(Dirofilaria immitis).* At present, this disease is not a problem in South Africa, but should it be imported unknowingly, and source of infection thus be established, the necessary mosquito vectors and a large susceptible population are present to complete the epidemiological chain. Once established, this disease has proven to be extremely difficult, if not imppossible to eradicate. In the U.S.A. the distribution and incidence of this disease has spread alarmingly during the last few years despite increased public awareness and advances in diagnosis, therapy and prophylaxis. The disease can be transmitted by 29 species of the genus Aedes, 12 species of the genus Anopheles, 14 species of the genus Culex and 6 species of the genus Mansonia. Many of these mosquitoes occur in South Africa and there is no reason why introduction of a source of infection will not lead to massive spread of the disease. Transmission by mosquitoes is biological and young adult worms reach the right side of the heart in 70 to 90 days. The total prepatent period of the disease is approximately $6\frac{1}{2}$ months, after which time live microfilaria are released by the adult female worms into the general circulation. The pathophysiology of this disease is variable depending on the number of adult worms present and their location. Most commonly the worms are present in the right ventricle and the pulmonary arteries. If present in moderate number, classic heartworm heart disease results, characterized by pulmonary hypertension as a result of endarteritis and obstructive fibrosis of the pulmonary vascular bed. This causes these normally highly distensible vessels to transform into a system of semi-rigid tubules. The result is pulmonary hypertension with progressive right ventricular enlargement leading eventually to right sided heart failure with its classical picture of peripheral oedema, ascites and hepatomegaly. The worms rarely, if ever, interfere with valvular function, but occasionally will localize in large number in the posterior vena cava and hepatic veins causing thrombosis and occlusion. This results in rapid onset of weakness haemoglobinuria, severe icterus and death. The clinical symptoms in early cases of classical dirofilariasis are increased appetite, loss of body weight, mild anaemia, non productive deep chest cough and occasionally haemoptysis and syncopic attacks.

Diagnosis:

The common method used to diagnose this disease is to examine a fresh drop of peripheral blood under a coverslip using the ten power objective. Motile microfilaria in varying numbers are visible. It should be born in mind that this method is only 80 per cent reliable because of the periodicity of the microfilaria, (being more prevalent and active during the late afternoon and early evening). Various methods, which are more accurate, are available, and entail concentration of the microfilaria by means of formalin precipitation and centrifugation or using various micropore filters. The microfilaria must be differentiated from those of *Dipetalonema reconditum,* a relatively harmless parasite living in the subcutaneous tissues. Occasionally dogs with advanced heartworm heart disease will have nagative peripheral blood, the reason for which is not known. In these cases one must rely on radiography for diagnosis. The classical radiographic picture in advanced heartworm heart disease is a) right ventricular hypertrophy, b) an enlarged pulmonary artery segment and c) large bore, non tapering and relatively radio dense pulmonary artery tree.

The treatment and prophylaxis of this disease consists of three phases: a) Destruction of adult worms using Thiacetarsamide – given intravenously for 3 consecutive days. b) Six weeks later a seven to ten day course of Dithiazanine is given orally to destroy microfilaria. c) 3 weeks later after checking peripheral blood is free of microfilaria, the dog is given Diethylcarbamazine daily in its food for prophylaxis against reinfestation. This must be given 365 days a year in tropical and sub-tropical areas and throughout the mosquito season in more temperate climates.

It should also be remembered that the treatment of the disease is not without danger, especially due to parasitic pulmonary emboli and multiple infarction. Older dogs with advanced heartworm heart disease are poor risks and should rather just be digitalized for the remainder of their life.

As you will realize, this disease is indeed a threat and if not looked for, could easily be missed. In view of the long prepatent period and periodicity of the microfilaria, I propose that all dogs imported from endemic and epidemic countries should be thoroughly examined at various intervals during their quarantine period and again three to six months later. Fresh wet blood preparations and possibly microfilaria concentrating techniques should be employed because many dogs are asymptomatic and yet still constitute a source of infection. It might also be wise to use mosquito proof netting on the quarantine cages and runs.

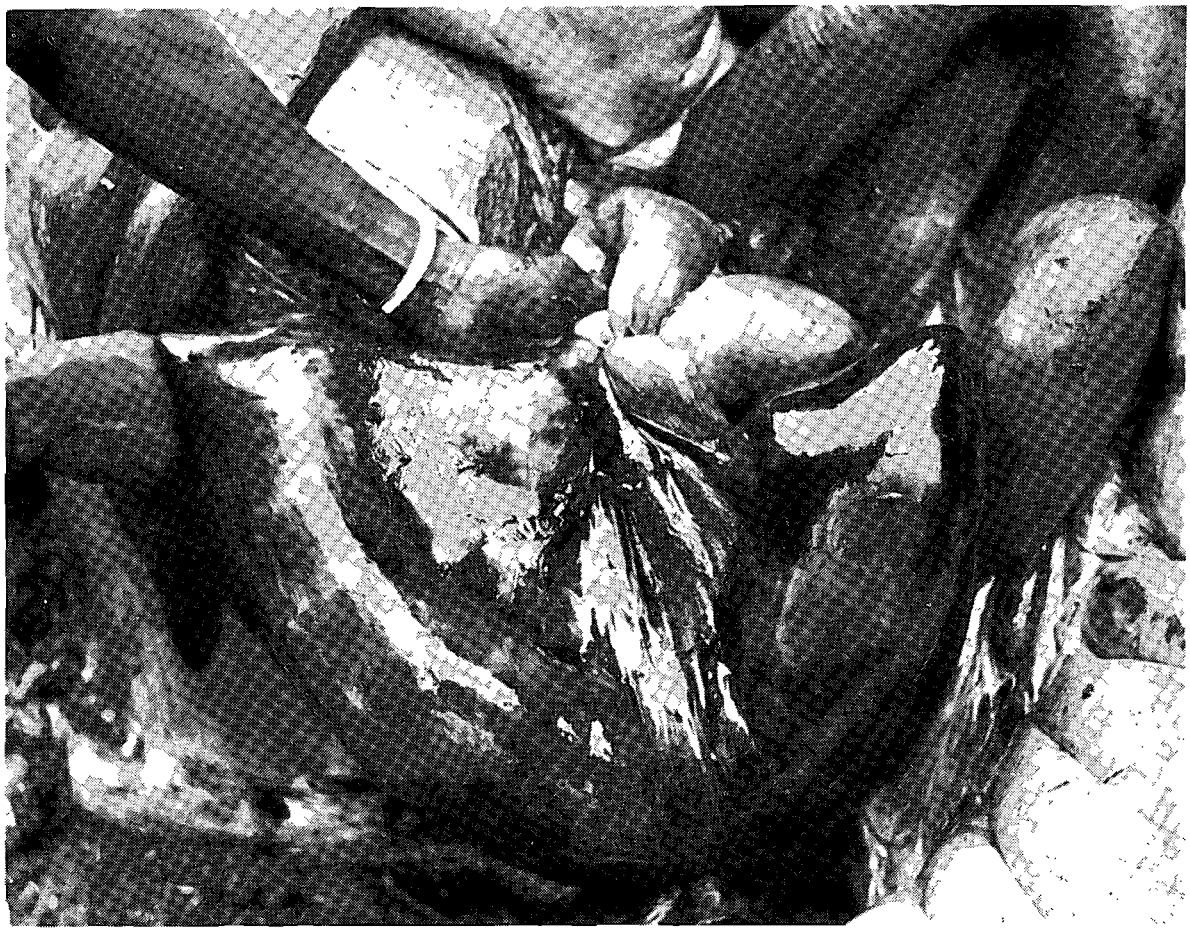
I believe that if these measures are followed, the risk of importing Dirofilariasis will be greatly reduced.

Yours faithfully,

ROY G. BENGIS, BVSc. MSc.

Department of Physiology and Biophysics School of Medicine
The University of Mississipi Medical Center
Jackson
Mississippi

FEATURE PAGE **TREFFERBLAD**



VOLVULUS IN A WHITE RHINOCEROS
(Ceratotherium simum)

On the 29th October 1974 an old female white or square-lipped rhinoceros *(Ceratotherium simum)* was found dead in the Kruger National Park. She had a 7-month old calf at heel. A necropsy was performed the next day.

On opening up the abdomen signs of an acute diffuse peritonitis were visible. The blood vessels of the stomach and intestines were distended and a large quantity of foul-smelling dark-coloured fluid with pus was found in the abdominal cavity. Advanced abdominal and intestinal tympany were present. As depicted by the above figure, a portion of the small intestine was twisted around its own axis, winding the mesentery taut to form total occlusion or strangulation of the lumen and blood vessels. In the above figure the affected portion was held by an assistant. The upper arm indicates the point where twisting, occlusion and strangulation took place. A diagnosis of volvulus was made.

The rhinoceros is supposed to be a species closely allied to the horse, both being members of the *Order Perissadactyla*. A similar layout of the lower alimentary system further corroborates this theory. The gross anatomical features which predispose volvulus in the horse, are therefore also present in the rhinoceros and must have some significance in the aetiology of the present case.

Contributor:
Dr V de Vos
National Parks Board
Skukuza
Kruger National Park

VOLVULUS IN 'N WITRENOSTER (CERATOTHERIUM SIMUM)

Op die 29ste Oktober 1974 is 'n ou witrenoster *(Ceratotherium simum)* koei dood gevind in die Nasionale Krugerwildtuin. Geen uitwendige tekens vir die oorsaak van dood kon vasgestel word nie. 'n Sewe-maande oue kalf was by haar. 'n Nadoodse ondersoek is die volgende dag uitgevoer.

Met die opening van die buikholte is tekens van 'n akute verspreide peritonitis waargeneem. Die are van die maag en dermkanaal was duidelik geswolle en 'n groot hoeveelheid slegreukende donkergekleurde vloeistof, gemeng met etter, is in die buikholte gevind. Die maag en dermkanaal was erg opgeblaas. Soos weergegee deur bostaande figuur, het 'n gedeelte van die dunderm met meegaande mesenterium 'n slag om sy eie as gemaak, om sodoende totale afsluiting van die dermkanaal en bloedvate te bewerkstellig. In bostaande figuur hou 'n assistent die aangetaste gedeelte vas. Die boonste arm van die assistent dui die plek aan waar verdraaiing en afbinding plaasgevind het. 'n Diagnose van volvulus was gemaak.

Die renoster is veronderstel om 'n naverwante spesies aan die perd te wees. Beide is dan ook lede van die *Orde Perissodactyla*. 'n Eenderse uitleg van die laer spysverteringsisteem verstrek hierdie teorie verder. Die algemene anatomiese eienskappe wat die perd tot volvulus predisponeer, is dus ook teenwoordig in die renoster en behoort betekenisvol te wees in die etiologie van die betrokke geval.

D V de Vos
Nasionale Parkeraad
Skukuza
Nasionale Krugerwildtuin

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Bold page number indicates senior or sole author
Vetgedrukte bladsynommer dui enigste of senior outeur aan

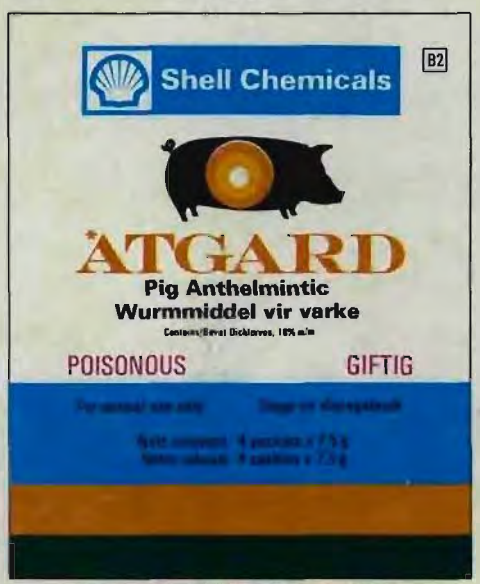
Hierdie varkie het mark toe gegaan.

Hierdie outjie het met rasse skrede gegroei tot 'n groot, spekvet otjie want geen parasitiese ingewandswurms het hom van sy opbouende kos beroof nie.

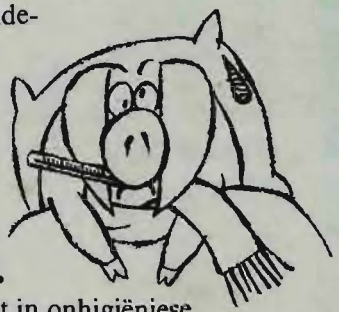
Feitlik elke kriesel van sy voedsel is omskep in onbelemmerde liggaamsgroei. Gevolglik was sy ontwikkeling vinniger en die koste, tot hy volgroeid was, laag.

Danksy die beplande behandeling met Atgard wurmmiddel vir varke tesame met higiëniese toestande terwyl hulle grootword, was dié snelle ontwikkeling moontlik.

Atgard is feitlik altyd 'n 100% doeltreffende doder van die drie soorte volwasse wurms wat hoofsaaklik by varke voorkom: rondewurms; en die klein knoppies- en sambokwurms wat dikwels nie raakgesien word nie.



Reg. No. G70, Wet 36/1947 Bevat: dichlorvos



Hierdie varkie het by die huis gebly.

Hierdie otjie het in onhigiëniese toestande groot geword en hy was ook nie doeltreffend ontwurm nie.

Gevolglik het hy sy eetlus verloor en stadiger gegroei. Sy derms en ander organe is beskadig soos die wurms deur sy liggaam beweeg het. En sy gesondheid is heeltemal verwoes.

Eers het hy baie gehoes en later het hy buikloop gekry. Die uiteinde – die ot het gevrek aan longontsteking (of was dit geelsug?)

Daarom het hierdie varkie nie die mark gehaal nie.

Hoe Atgard varke meer winsgewend by die mark kry as enige ander ontwurmer.

Atgard is die enigste ontwurmer wat feitlik altyd 100% doeltreffend is teen die drie soorte volwasse wurms wat varke oor die algemeen in Suid-Afrika besmet.

Atgard word maklik toegedien – dit word eenvoudig in die dier se kos gemeng.

Soos die korreltjies deur die dermkanaal beweeg word wurmdodende dichlorvos stadig vrygelaat.

Die korreltjies is onverteerbaar en word meestal binne 96 uur uitgeskei.

En omdat dit heeltemal veilig is, kan Atgard vir die ontwurming van klein varkies gebruik word asook in die sog kort voor geboorte om besmetting van wurmeiers by die werpsel tot 'n minimum te beperk.

U teel hulle. Ons beskerm hulle.

Shell Chemikalieë

Landbouchem Afdeling.

Nou verkrygbaar by u Koöperasie.

Partnership 121/A