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# TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINERE VERENIGING

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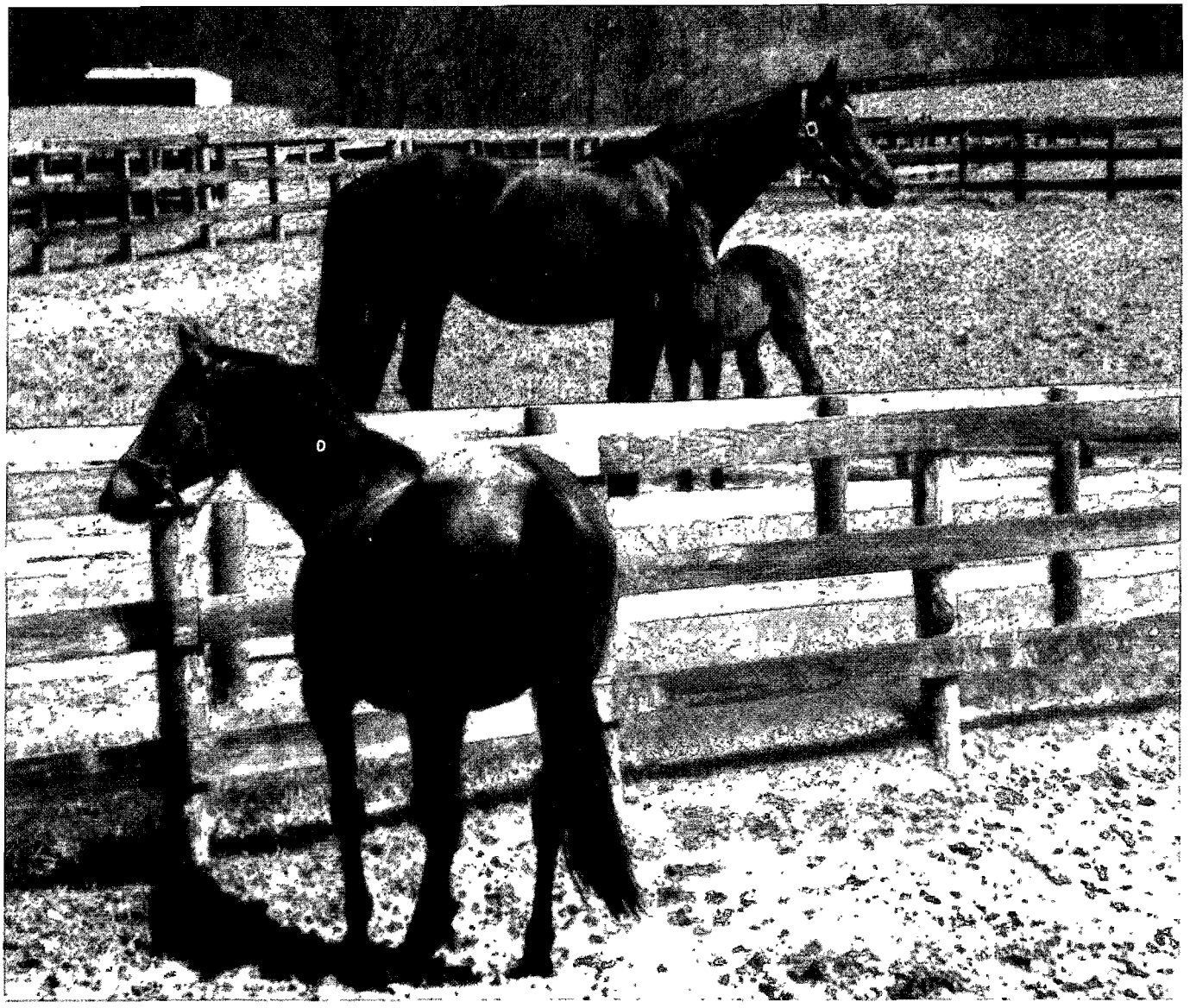

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## EDITORIAL

# THE SHORTAGE OF VETERINARIANS

More than twenty years ago this Association reviewed the situation and pertinently pointed to the shortage of veterinarians and the effect thereof on the profession and the country. Gradually our only training facility was increased to take in 30 (1955), 40 (1962) and 45 (1963) students per year. In 1964 the shortage still existed and the then President of the S.A.V.M.A. wisely mooted the establishment of a further training facility to augment the existing one and provide training facilities for the increasing number of students wishing to make a career of veterinary science.

In response to a request the Association in 1968 submitted a comprehensive memorandum to the Minister of Agriculture regarding the shortage of veterinarians and the need for extended training facilities. A year later the Cabinet accepted Prof. Mönnig's recommendations to expand the existing Faculty rather than establish another Faculty as recommended by the Association. Now, six years later, the Minister has announced that 90 students will be admitted to the Faculty of Veterinary Science of the University of Pretoria as from 1976. At the same time it is learnt that the B.V.Sc. course will be extended from 5 tot 5½ years, and that a combined interethnic facility for the training of a limited number of Black dentists, medical practitioners, and veterinarians is to be established near Pretoria.

In the meantime the shortage of veterinarians is becoming more acute than ever in all walks of professional activity. The present shortage is authoritatively stated to be around 118 (a figure which is undoubtedly conservative) while a projected shortage of 297 by 1980 and 734 by 1985 is to be anticipated if 40 veterinarians graduate every year and the professional erosion factor is taken at 4%. If 80 graduates p.a. could be achieved from 1980, the shortfall would still amount to 253 in that year and 529 by 1985.

It is abundantly clear that presently projected training programmes cannot help to eliminate the alarming shortage of veterinary manpower that lies ahead. Importation on a large scale of foreign graduates and/or the creation of further training facilities are obvious answers to the problem. The intake of students at any single school must be limited to that number which can adequately be supplied with the necessary quality, quantity and variety of clinical teaching material which the readily accessible environment can supply. As is the case with Australia and other countries, South Africa has an essentially extensive livestock industry. It cannot hope to provide, at any one centre, the concentrations of teaching material for the large numbers of students equivalent to those accommodated by schools in the intensively developed countries of Western Europe and North America. The suggestion that as many as 120 students be admitted annually to the existing Faculty is frankly inconceivable.

The veterinary profession has an undoubted role and responsibility to help feed the growing millions, and South Africa can ill afford the stock losses which are largely preventable by an adequate veterinary service. Such losses have been calculated at about R359 million per annum.

## REDAKSIONEEL

# DIE TEKORT AAN VEEARTSE

Reeds meer as twintig jaar gelede het hierdie Vereniging ondersoek ingestel na, en pertinent gewys op die tekort aan veeartse en die invloed daarvan op die beroep en die land as geheel. Geleidelik is ons enigste opleidingsfasiliteite aangepas om 30 (1955), 40 (1962) en 45 (1963) studente in die tweede studiejaar op te neem. Teen 1964 het die tekort nog bestaan – en dit te midde van toenemende getalle studente wat veeartsenykunde as loopbaan gekies het – en die toenmalige President van die S.A.V.M.V. heel verstandiglik voorgestel dat 'n verdere opleidingsfasiliteit ter aanvulling van die bestaande ingestel word.

Volgende op 'n versoek het die Vereniging 'n deurtastende ondersoek ingestel en in 1968 'n omvattende memorandum by die Minister van Landbou ingedien. Hierin is die tekort aan veeartse en die vraag na opleidingsfasiliteite vir meer studente uiteengesit met 'n sterk gemotiveerde aanbeveling tot die stigting van 'n tweede fakulteit elders in die Republiek. 'n Jaar later, en in weerwil hiervan, aanvaar die Kabinet die aanbeveling van Prof. Mönnig om liewers die bestaande Fakulteit aan die Universiteit van Pretoria uit te brei.

Nou, ses jaar later, is deur die Minister van Landbou aangekondig dat tot 90 studente vanaf 1976 tot die tweede studiejaar aan die bestaande Fakulteit toegelaat sal word. Tegelykertyd word verneem dat die B.V.Sc.-kursus na 5½ jaar verleng sal word. Intussen is ook verneem dat planne vir die stigting van 'n gekombineerde fakulteit vir die opleiding van Swart veeartse, tandartse en geneeshere vêr gevorder is.

Intussen word die tekort aan veartse in alle sfere van professionele aktiwiteite gaandeweg al hoe meer nypend. Gesaghebbend word die huidige tekort konserwatief op 118 gestel, terwyl die geprojekteerde tekort teen 1980 op 297 en teen 1985 op 734 te staan kom indien 40 nuwe graduande per jaar deur die Veeartsraad geregistreer word en 'n professionele erosie faktor van 4% in berekening gebring word. Indien 80 bykomstige veeartse elke jaar van 1980 vir registrasie aanmeld sou daar in daardie jaar nog 253, en in 1985 nog 529 te min veeartse in die R.S.A. beskikbaar wees.

Dit is nou maar eenmaal onmiskenbaar en duidelik so dat die huidige en beplande opleidingsprogram nie daarin kan slaag om die kritiese tekort aan veartse wat reeds bestaan en in erger graad voor die deur lê, uit te wis nie. Grootskaalse immigrasie van buitelandse veeartse en/of die onmiddellike instelling van verdere opleidingsfasiliteite is die enigste wyses waarop die tekort uit te wis.

In sekere kringe is daar sprake van tot 120 studente by die bestaande Fakulteit op te neem. Die opname van studente by enige enkele veeartsenyskool word noodwendig beperk deur die hoeveelheid, kwaliteit en verskeidenheid van opleidingsmateriaal wat vanuit die redelik-bereikbare omgewing getrek kan word, aangesien dit grondliggend tot 'n bevredigende standaard van opleiding is. Soos in die geval van Australië en ander sulke lande, is die veebedryf in Suid-Afrika essensiëel ekstensief van aard. Die bedryf om enige enkele sentrum kan dus nooit die konsentrasie van opleidingsmateriaal voorsien vir die doeltref-

The consequences of a shortage of veterinarians other than a direct financial loss in preventable acute and erosion diseases, are numerous but a few examples are sufficient to emphasise the dire straits in which we find ourselves. There are insufficient veterinarians to effectively control our notifiable diseases, to undertake new research programmes, to prevent unhealthy competition between the more highly paid posts in the private sector and the State, to allow for expansion in private practice, both urban and rural and to fill all the numerous positions available in other spheres such as Commerce, Teaching and Public Health.

This must lead to inevitable substitution by other professional, technical and even lay men, where lower standards may prevail, to the detriment of the livestock industry, our companion animals, our Country – and its people.

Every effort should be made to recruit *adequate* staff in the first instance for the existing Faculty to make it strong and viable and then for a second faculty so that in effect, there will be a doubling of academicians in South Africa with all the potential this implies. The large turnover in staff at the Faculty is disquieting and the reasons, – whether financial or otherwise, should be fully investigated and corrected. The opportunities for promotion in the academic field with two faculties will be a great incentive in attracting potential teachers at present in other spheres of employment, let alone the siting of the new faculty in an environment with it's own peculiar problems and challenges. There is too, we feel, a reservoir of trained academicians in other employment both here and overseas who, for one reason or another, have left the Faculty but who will return to an academic career in other circumstances.

While staff will undoubtedly be attracted away from the Faculty, the Veterinary Research Institute and other branches of the profession to join a new faculty, the reward of the long term benefits to be gained in the interests of the national agricultural economy is a price we feel that can and must be paid.

The Association makes no apology for having an opinion on this important matter of veterinary education. An honest opinion ought to have a stimulating function. There is no desire to raise this matter purely for the sake of controversy, and the Association has for many years taken a definite and responsible point of view. There can surely, at this late stage, now be no more doubt about the urgent need for the immediate establishment of another veterinary faculty.

fende opleiding van die groot getal studente wat in die intensief-ontwikkelde lande van Wes Europa en Noord Amerika se veeartsenyskole aangetref word nie.

Die Vereniging bied geen verskoning aan vir die feit dat hy menings oor hierdie belangrike aangeleentheid huldig nie. 'n Eerlike mening behoort stimulerend te wees. Daar is geen begeerte om bloot terwille van polemiek te polemiseer nie. Die Vereniging het oor baie jare 'n duidelike standpunt ingeneem.

Die professie het 'n onbetwisbare rol om te speel en dra sekere vaste verantwoordelikhede om die toenemende bevolking te voed en te kleed. Die Republiek kan kwalik die veeverliese bekostig wat as gevolg van ontoereikende veeartsenykundige dienste ontstaan. Sodanige verliese, wat grootliks vermybaar is, word geraam op R359 miljoen per jaar.

Bo en behalwe die direkte geldelike verliese wat volg op voorkombare skute en erosie-siektes is daar verskeie ander gevolge van 'n tekort aan veeartse. Enkele voorbeelde is egter voldoende om die haglike posisie waarin ons onsself bevind, toe te lig. Daar is onvoldoende veeartse om ons aanmeldbare veesiektes doeltreffend te beheer, om nuwe navorsingsprojekte te onderneem, om ongesonde mededinging tussen die beter besoldigde betrekkings in die private sektor en die Staatsdiens te verhoed, om uitbreiding van privaatpraktyke in die platteland sowel as die stede toe te laat, en om die getalryke beskikbare poste in ander werksfere soos die Industrie, Opvoeding en Volksgesondheid te vul.

Noodwendig lei so 'n toestand tot vervanging deur ander professionele, tegniese en lekepersoneel, en die laer standaarde wat as gevolg daarvan kan ontstaan is tot nadeel van die veebedryf en die troetel- en vermaakdiere in ons land – en derhalwe van ons volk.

Alle moontlike pogings moet aangewend word om in die eerste plek toereikende personeel vir die huidige Fakulteit te rekruteer sodat dit sterk en lewensvatbaar word. Dieselfde geld vir 'n tweede fakulteit sodat daar as't ware 'n verdubbeling van veeartsenykundige akademici ontstaan – met al die kragdadigheid wat dit impliseer. Die groot omset van personeel aan die huidige Fakulteit is verontrustend en die redes daarvoor, t.w. geldelik of andersins, behoort deeglik nagegaan en reggestel word. Wanneer twee fakulteite bestaan sal die geleenthede vir bevordering in die akademiese sfeer as groot aansporing dien vir die werwing van potensiale dosente wat huidiglik elders in diens staan. Dieselfde geld vir die plasing van 'n tweede fakulteit in 'n omgewing met sy eie besondere probleme en uitdagings. Daar is na ons mening ook 'n resevoir van opgeleide akademici wat beide hier te lande en in die buiteland elders in diens staan en wat die bestaande Fakulteit om een of ander rede verlaat het maar wat onder ander omstandighede weer 'n akademiese loopbaan sal opneem.

Personeel sal ongetwyfeld van die bestaande Fakulteit, die Navorsingsinstituut en ander vertakkings van die beroep weggetrek word om by 'n tweede fakulteit aan te sluit. Na ons mening kan en moet hierdie prys betaal word indien die langtermynvoordele vir die land se veebedryfekonomie ondervind moet word.

Op hierdie laat stadium kan daar sekerlik nou by niemand nog enige twyfel bestaan oor die dringende noodsaaklikheid vir die vroegtydige oprigting van 'n tweede blanke veeartsenykundige fakulteit in die R.S.A. nie.

Jl S.Afr. vet. Ass. 46(3) 221-225 1975

ADDRESS TOESPRAAK

# MEDICAL ASPECTS OF SOME ZOONOSES

J.H.S. GEAR*

The subject the zoonoses is of great mutual interest to the veterinary and medical professions. In a recent book Thomas Hull lists about 200 diseases transmitted from animals to Man. It is clearly not possible to review in a worthwhile manner so extensive a subject in one lecture. Instead a few examples from our own experience of these conditions in Southern Africa will be taken to illustrate the importance of some of the animal diseases transmissible to Man.

*Virus Infections*

The classical example of an arthropod-borne virus disease is yellow fever which was the first disease of Man shown to be caused by a virus and proved to be transmitted by a mosquito. This investigation was carried out by a team under Major Walter Reed sent to Cuba during the American-Spanish War in the first years of this century to study this serious threat to the American troops operating on the island.

Further studies have revealed that yellow fever in Africa infects monkeys living in the forest and that it is transmitted amongst them by a forest mosquito *Aedes africanus*. When these monkeys raid the village gardens and crops, they may be bitten by another mosquito species *A. simpsoni* which may then be infected and transmit the infection on to Man and so may initiate a human epidemic in which interinfection is spread by *A. aegypti*.

Yellow fever, once was one of the greatest threats to Europeans whose occupation brought them to West Africa and other endemic areas. This fear has been lifted by the development of a vaccine by a team headed by Dr. Max Theiler, a South African working in the Laboratories of the International Health Division of the Rockefeller Foundation. It is one of the best human vaccines so far produced and protects over 95% of those inoculated.

Yellow fever does not occur in the Republic of South Africa and we will begin our story of the infections in this country by recalling that on Wednesday, 11th April, 1951, a valuable bull on the farm Rietvlei, belonging to the Social Welfare Department of the Johannesburg City Council suddenly went beserk, blundered blindly through a barbed wire fence, bellowed, collapsed and expired.

A post mortem examination was carried out by Dr. P.J. Meara of the Council's Veterinary Department, assisted by four of the farm hands. Two of them held the legs and did not handle the incised tissues. The other two did get their hands contaminated with blood in opening the carcase. Dr. Meara examined the organs. Drs. A.A.L. Albertyn and R.K. Loveday, also of the Veterinary Department, arrived on the scene two hours later. They also handled the organs. None of them used gloves.

Dr. Meara reported on his findings as follows:

"The intestinal mucosa, especially of the small intestive and caecum, was reddened. The contents were bloody. The wall of the gall bladder was oedematous and bloody. Focal necrosis of the hepatic lobules was evident throughtout the liver. Multiple petechial haemorrhages, ecchymosis and extravasations were present under the epicardium, endocardium, pleura and peritoneum".

Four days later the three veterinarians and two of the farm hands suddenly became acutely ill, with rigors, headache, sore congested eyes, pain in the limbs and back and slight nasal bleeding. They also had loss of appetite and slight nausea. The temperature chart showed a biphasic fever, the first bout lasting 3 days followed by a remission when the patients felt better followed by a recrudescence of the symptoms and fever lasting one to two days, after which the patients felt better, except for weakness. Convalescence was rapid.

We were notified of this outbreak of illness by Dr. Mundel, Medical Officer of Health of Johannesburg and a portion of the bull's liver which had been stored in deep freeze was sent to the Institute. A suspension was prepared and inoculated intracerebrally and intra-abdonimally into mice. These mice all died 2-4 days later and post mortem examination revealed a congested and friable liver which, on microscopic examination, showed that most of the parenchymal cells were degenerate. Their cytoplasm showed eosinophilic masses not unlike the Councilman bodies found in the livers of the human beings dying of yellow fever. The nuclei show eosinophilic inclusions. This pathological picture is similar to that produced by the virus of Rift Valley fever, which was suspected as the cause of the outbreak.

A virus producing similar lesions in mice was isolated from one of the patients and serological tests proved that the other patients had been infected with the same virus, proved to be that of Rift Valley fever. This infection had not hitherto been known to occur in South Africa and its source was an intriguing question.

At first it was suspected that the virus had been introduced by air traffic from East Africa where Rift Valley fever was first discovered. This suspicion was discounted when it was found that the bull had died towards the end of a widespread epizootic affecting the Western Orange Free State, North Western Cape

Read at the 25th Annual General Meeting of the Cape West Branch, SAVA, August 1974

*Poliomyelitis Research Foundation, Johannesburg.

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and the South Western Transvaal, in which thousands of lambs had died and nearly all pregnant ewes had aborted. Serological studies revealed that his disease was Rift Valley Fever.

At the same time as the occurrence of this epizootic, many cases of an acute febrile illness occurred in human beings whose occupation brought them into direct contact with sick animals. Most of them had cut open and handled the viscera of sheep. None died, but several developed a chorio-retinitis with a fluffy exudate in the retina associated with defective vision. Their illness was proved to be Rift Valley fever by the isolation of virus from several typical cases and by serological tests.

In the autumn of 1953 the disease once again appeared in the Luckhof district of the Orange Free State. In studies carried out in the field, *A. caballus* and *Culex theileri*, two mosquitoes most prevalent in the Panveld were incriminated as the vectors of the infection in this region. Serological surveys revealed that a polecat, cattle, sheep and buck had been infected but no other animal reservoir of the infection was detected.

This outbreak was the beginning of our arthropod borne virus research programme. In the 25 years since then our team, headed by Dr. B. McIntosh, has identified 30 immunologically distinct arboviruses occurring in this region. Ten are known to cause infection in Man and amongst these in addition to Rift Valley fever, are chikungunya virus, Sindbis and West Nile virus, all of which cause a dengue-like illness characterized by painful stiff joints, headache and a biphasic temperature chart.

Chikungunya fever was first described in the Newala Province of Tanzania and was given the local name meaning "painful bent stiff joints." The second epidemic affected the population of Eastern Transvaal and many visitors to the Kruger National Park from the Witwatersrand at Easter time in 1957. Studies since then have shown that monkeys and baboons may be infected in nature but it is possible that like man, they are involved only incidentally in a cycle of infection involving other animals in the bush: this has not been elucidated.

Immunity serveys undertaken have revealed that chikungunya virus is essentially a tropical infection. On the other hand Sindbis and West Nile infections are prevalent over the whole of South Africa and just as prevalent on the highveld as in the sub-tropical coastal regions. It has been shown that in nature both infections occur in wild birds being spread amongst them by mosquitoes, particularly *C. univittatus* and, accidentally almost, Man and his domestic animals may occasionally be infected. In Man the infection results in a dengue-like illness.

We all will recall that last summer and autumn South Africa had regular heavy falls of rain which resulted in extensive floods in the Orange River Valley and the surrounding territories. These conditions favoured the proliferation of mosquitoes and thousands of cases of a dengue-like illness were reported from the region. Studies carried out by Dr. McIntosh and his colleagues proved that cases were due to West Nile virus and that the source of infections for the mosquitoes were the numerous birds, in particular the sparrows, weavers and thrushes.

In 1949 an expedition sponsored by the Botswana Government set off from Maun to carry out a survey of yellow fever in the Ngamiland swamps. The day before it set out, a small dog was noted to be lying in the corner of the hotel lounge.

When the expedition returned three weeks later, we learnt that this dog was a stray which had come from none knew where. It had got into a fight with two prize Scottish Terriers owned by the district medical officer, and had then bitten the district commissioner and several other people. After this violent encounter and suspicious behaviour it was killed and its brain was sent to Onderstepoort where it was proved to be infected with rabies virus. In the following year, 1950, dog rabies was recognized in the Northern Transvaal.

It appears that there are two ecological forms of rabies in South Africa. One mainly involving canidae extending from the Northern Transvaal east of the Drakensberg, southwards into Natal and the Eastern Cape Province, the other mainly involving the viveridae and in particular *Cynictis penicillata*, the yellow mongoose or rooi meerkat. Most human cases have occurred following the bites of this animal.

*Chlamydial Infections:*

My next story concerns our detectives. They were hot on the trail of the baddies engaged in a lucrative racket and indeed had caught their birds when nearly all the members of the squad were afflicted by an illness characterized by a sudden onset with rigors, severe headaches, pain in the eyes and muscles, and cough. An X-ray examination showed a diffuse pneumonitis suggestive of viral pneumonitis. When the history of the infection was obtained the diagnosis was obvious. They had psittacosis contracted from South American parrots which were fetching a high price on the black market. This is only one of several such outbreaks: a recent one involved parrots coming in through the Cape Town docks.

Another one involved the contacts of Australian finches imported through Durban. These birds had a certificate to say that they had been quarantined the regulation 6 weeks before setting out on their sea journey. There was a suspicion that another batch of birds was sent in place of those that had been quarantined. However, it is more probable that the close, confined and overcrowded quarters on board ship favoured the reactivation and recrudescence of a quiescent infection and hence the outbreak in their human contacts in Durban.

In addition to these outbreaks, cases are regularly admitted to the Johannesburg Fever Hospital. The diagnosis on admission is usually virus pneumonitis, but becomes clear firstly from the history of contacts with birds and later from the results of serological tests, usually the complement fixation test. The birds most commonly involved are budgies, but cases have followed contact with pigeons and now we know that most wild and domestic birds may be a source of infection.

These patients respond well to treatment with tetracycline and usually make an uneventful recovery. However, there is one complication to which attention was called recently and which has ended fatally. It is chlamydial endocarditis superimposed on valves previously damaged by rheumatic fever.

*Rickettsial Infections:*

This takes me on to my next story. A medical scientist, while on holiday on a farm, carried out post-

mortem examination of lambs which had died. About three weeks later he developed fever and then became jaundiced with other signs of liver damage. It was concluded that he had infectious hepatitis, but his fever did not come down when the jaundice became apparent as is usual in cases of infectious hepatitis, but continued for several days. In the following months he had several recrudescences of fever, each lasting several days. He then developed signs and symptoms of subacute bacterial endocarditis but no bacteria were isolated from repeated blood cultures. However, the diagnosis became clear when his complement fixation test for *Rickettsia burneti* became positive about three weeks after the onset of his illness, attained high titres of 1:3200 and remained at his high level indicative of a continuing active infection from which he died after an operation to replace his damaged valves.

Rickettsial endocarditis is a rare complication of Q fever and most cases have been recognized in England and a few in Australia. Only two to my knowledge had been recognized in South Africa. Yet Q fever is the commonest rickettsial disease affecting Man in this country. It is so common and prevalent that most South Africans acquire the infection from contact with and inhalation of the dust emanating from tick infested cattle in childhood and usually before the age when rheumatic fever most frequently occurs. Perhaps this time relationship accounts for the relative rarity of this form of endocarditis in South Africa.

The disease escaped notice in South Africa until after World War II when a large number of susceptible immigrants arrived from Britain and North Western Europe. Most of them worked in cities and then took their first annual leave, some on farms. Soon after returning from leave many of them became ill with an illness charactersized by high fever, loss of appetite, sore throat, headache, muscle pains, and because their medical attendant suspected typhoid fever they were admitted to the Fever Hospital. On examination' it was noted that their eyes were injected, their face suffused. But their abdomen were not distended as is seen in cases with typhoid fever. The true diagnosis of Q fever was condirmed by the positive results in complement fixation tests. *R. burneti* is relatively resistant to physical and chemical agents, but adequate pasteurization of milk reduces its infectivity and relatively few cases can be traced to drinking of infected milk although this is one route of infection. However, it is clear Q fever is a zoonosis and Man is only incidentally and sometimes accidentally infected in the course of his duties.

The next most widespread rickettsial infection in South Africa is tick bite fever, the form of tick bite fever occurring in Southern Africa. It is caused by *Rickettsia conori var pijperi* and transmitted by the bite of larval ticks which, unlike adult and nymphal ticks, are so small that they are not felt walking on and attaching to the skin. The clinical illness is characterized by a local lesion, a papule with a central black eschar which develops at the site of the infecting bite, regional lymphadenitis, fever and a maculopapular rash which characteristically involves the palms of the hands and soles of the feet and the face. Treatment with tetracyclines results in a dramatic amelioration of the patient's condition which begins 48 hours after starting therapy. The temperature falls, the rash rapidly fades and the patient feels and is better.

Studies carried out in our laboratories revealed that veld rodents, in particular *Rhabdomys* and *Otomys*, are infected in nature and may play a part in infecting lines of ticks not hitherto infected but the infection may be passed from one generation of ticks through the eggs to the next, all stages of which are capable of transmitting the infection. This hereditary transmission from one generation to another may continue indefinitely and so there is no need for a mammalian reservoir to maintain tõe infection, but clearly tick bite fever is a zoonosis.

*Leptospiral Infections:*

Then I come to my next anecdote. A girl was given a puppy which she took for walks. A boy admired the girl very much and to show his affection, he embraced her puppy and it returned the embrace by licking their faces. Several days later they both were admitted to Johannesburg Fever Hospital, suffering from intense headache. On examination it was noted that both of them had intensely congested eyes, and muscle pain, especially in the calves, and fever which had shown a biphasic chart and it was during the second wave of fever that they had developed signs of central nervous system involvement with meningoencephalitis. On lumbar puncture the cerebral spinal fluid showed pleocytosis. The urine showed evidence of kidney involvement and the liver function tests, evidence of parenchymal cell damage. Serological tests revealed that the cause of their illness was *Leptospira canicola*. Serological tests on the puppy showed high titres against this organism and there is little doubt that it was the source of infection, probably through the media of its urine.

About the same time several other human cases of leptospirosis were recognized and in each case the source of infection was traced to the dogs of the households. This infection is not common but clearly more work is needed to define the prevalence of this and related infection.

*L. icterohaemorrhagiae* has not been found in the rats of Johannesburg. It is a common parasite of the rats of Cape Town and Durban.

*Trypanosomiasis:*

My next story is about a party of hunters who went to hunt lion and buffalo in Ngamiland. Buffalo blood is the favourite food of *Glossina morsitans*, the tsetse fly and transmitter in that region and over much of South Eastern Central Africa of *Trypanosoma rhodesiense*, the cause of Rhodesian sleeping sickness and *T. brucei*, the cause of ngana. It may be recalled that Sir David Bruce, then a medical officer in the RAMC dicovered that ngana was caused by a trypanosome (now named *T. brucei* in his honour) while investigating this disease in Zululand. He made this discovery before Ross proved that malaria was transmitted by mosquitoes. It was a milestone in the history of tropical medicine.

Two weeks later, after the party of hunters returned to South Africa, two members took ill suddenly. They were brothers; one was treated with Bayer 205 and was cured. The other who was a minister in a church which did not believe in medicine, refused treatment and in spite of pleadings and threats' remained ada-

mant in his refusal. He said he always knew he would be put to the test and was confident that his faith would see him through. It seemed to, for he went back to his preaching saying the doctors said he would die, and here he was back in the pulpit, but in the end he did die five months after contracting the infection.

Since then cases have been regularly seen in the Johannesburg Hospital, most of whom have contracted their infection in the Ngamiland swamps. If treatment is begun early one can always guarantee a cure, but not always. One patient who showed the early signs of trypanosomal infection including the primary lesion trypanosomal chancre, enlarged glands and fever, in spite of treatment died three days after his admission to hospital of disseminated intravascular coagulopathy a complication of endotoxaemia and of infections associated with the circulation of numerous foreign particles either of viruses, rickettsial, bacterial or malarial parasites and as we now know, numerous trypanosomes in the circulating blood.

Nearly all patients treated with Suramin are cured. If treatment is delayed usually because the diagnosis has not been made, the outlook is not so good but even then with appropriate additional treatment to deal with the central nervous system involvement, many cases recover.

Lest I should be accused of spreading alarm and despondency amongst the veterinary profession in regard to the dangers of the infections of animals, I would like to end by noting that vaccines have been developed to protect its members against Rift Valley Fever, rabies and several other infections. But perhaps more important, protection can be given by the simple expedient of wearing gloves when doing post mortem on animals which have died mysterious deaths.

*Toxoplasmosis:*

For my last story, I quote the nursery rhyme:

This is the priest all shaven and shorn
Who married the man all tattered and torn
Who kissed the maiden all forlorn
Who milked the cow with the crumbled horn
Which tossed the dog which chased the cat
Which killed the rat which ate the malt
Which lay in the house that Jack built.

Put in modern prose: Jack married Jill and moved into a new house and they were given a new kitten which killed and ate a toxoplasmic mouse and then Jill became pregnant and became infected from her cat and this is perhaps one reason why congenital defects due to toxoplasmosis are more common than might be expected by chance.

*Conclusion:*

Finally I am grateful for this opportunity of expressing my gratitude and that of my colleagues in the South African Institute for Medical Research and in the Laboratories of the Poliomyelitis Research Foundation for the co-operation and help we have received, in my case for a lifetime, from members of the veterinary profession. Many of our most rewarding research programmes have been undertaken with their collaboration and these have all been concerned with animal diseases transmissible to Man and the medical aspects of zoonosis.

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# BOOK REVIEW

# BOEKRESENSIE

## PATHOGENIC CLOSTRIDIA

MAX STERNE & IRENE BATTY

Butterworths, London & Boston 1975

pp viii +144; Figs 1, Plates 7 ( 3 in colour) Tales 12 (No price stated)

This small yet highly informative book is intended to provide field veterinarians and medical and science graduates with selected up to date and expert information regarding this important group of bacteria which are responsible for gas gangrene, enterotoxaemia, infections of hepatic origin and neurotropic intoxications. It deals specifically with data necessary for an appreciation of the importance of proper selection and transportation of samples, and provides information to examining laboratories concerning appropriate investigations for the detection of potentially pathogenic clostridia or their products in such samples. Great emphasis is rightly placed upon correct interpretation of findings in view of the ubiquitous distribution of the clostridia in soil, ingesta, dejecta, cadavers of animals and even in the tissues of normal animals after slaughter.

The chapters on the role of clostridial infections and the criteria for diagnosing such infections form an essential background to those dealing more specifically with the selection, preservation and examination of specimens. The figures, tables and plates are excellent and invaluable. (There is, however, an incorrect reference on page 24 to Table 2 instead of Table 3).

This publication will be of inestimable value to practitioners, laboratory workers and public health personnel alike.

Max Sterne is well known in South Africa. He was one of the first graduates of the Faculty of Veterinary Science of the University of Pretoria and worked for many years in the Veterinary Research Institute at Onderstepoort before taking up a position with Wellcome Laboratories.

L.W. vd H.

Jl S.Afr. vet. Ass. 46(3) 227-232 1975

REVIEW OORSIG

# AFRICAN SWINE FEVER: AN EPIZOOTIOLOGICAL REVIEW WITH SPECIAL REFERENCE TO THE SOUTH AFRICAN SITUATION

A. PINI* AND L.R. HURTER**

SUMMARY

The most important characteristics and the distribution of the viruses of African swine fever and hog cholera are reviewed. Both viruses were probably present simultaneously in South Africa in the first two decades of the century. While hog cholera was eradicated by 1918, African swine fever persists to the present day because it has a different epizootiology.

The role played by wild pigs and the argasid tick (*Ornithodoros moubata porcinus*) in the epizootiology of African swine fever is discussed and an account of the outbreaks of the disease in South Africa from 1926 to 1974 is given. It appears that the disease in the Transvaal has had a cyclic occurrence.

## INTRODUCTION

After an interval of approximately 10 years during which no cases of African Swine Fever (ASF) were recorded, the disease has re-appeared in the domestic pig population of the northern, eastern and western Transvaal. Between May 1973 and March 1974, 18 outbreaks were recorded, all within the enzootic area.

In South Africa the control of ASF since its first recognition in 1926 has been based on the slaughter of all infected and in-contact pigs, the confinement of swine in fenced premises and the restriction of the movements of pigs and pig products from, to and through the proclaimed area. It appears that these measures were successful in containing the virus in the natural reservoirs, namely the wild pigs and the argasid tick *Ornithodoros moubata*. From the epizootiological information available and under the present circumstances, the eradication of ASF appears to be impossible; it could only be achieved by an ecological transformation of the entire enzootic region, leading to the elimination of the natural reservoirs of the virus.

This paper is a review of the more recent findings on the epizootiology of ASF and gives an account of the outbreaks of the disease in South Africa from 1926 to 1974. The presence of hog cholera (HC) in the country at the beginning of the century is also discussed.

### *The Distribution and Characteristics of the Viruses of ASF and HC*

ASF is a highly pathogenic disease of domestic pigs characterized by degenerative changes of the lymphoid tissue and vascular system leading to extensive haemorrhages. The symptoms and macroscopic lesions of ASF can be differentiated only with extreme difficulty from those of HC; where the two diseases occur simultaneously a differential diagnosis is obtained by cross immunity tests in pigs or by other laboratory procedures.

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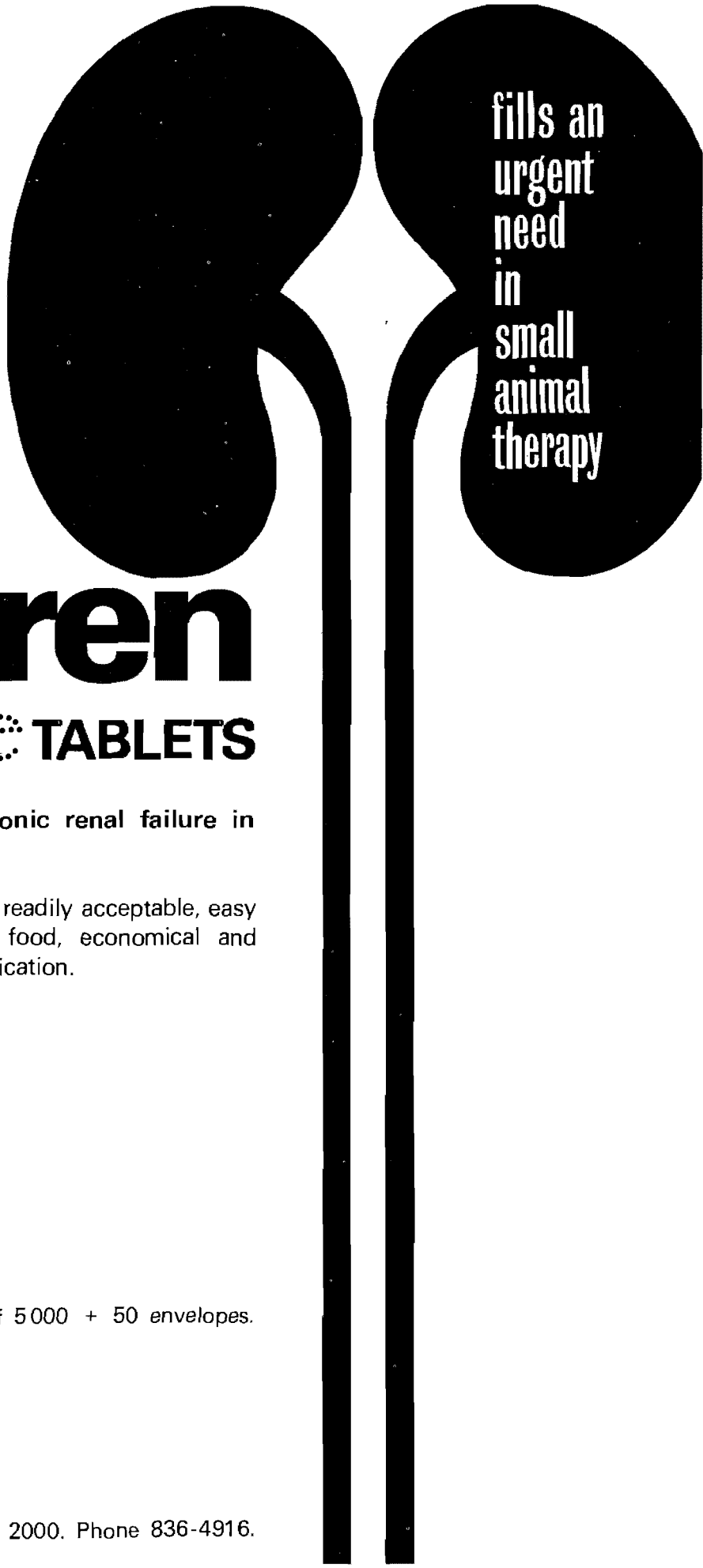
HC is indigenous to the U.S.A. and it is believed that originally a wild animal with a limited geographical distribution acted as the reservoir of the virus[24]. In the middle of the last century the disease began to spread, initially insidiously and then very rapidly, in the domestic pig population of the U.S.A. Between 1862 and 1887 it reached England and later became established all over Europe. Since then HC has been one of the most serious problems of the world pig industry.

ASF, however, is indigenous to the African continent south of the Sahara, where wild pigs are reservoirs of the virus. The disease made its appearance at the beginning of this century[14] when the balance between its natural hosts and the infectious agent was altered by the introduction of domestic pigs into Africa. The virus has now escaped from the African continent and has become established in Portugal and Spain. The disease has also occurred in France, Italy and, more recently, in Cuba.

HC and ASF viruses are clearly distinguishable in morphology, chemical composition and antigenic characteristics.

HC is an RNA virus with essential lipids and a diameter of 40nm. Its infectivity is destroyed by treatment with ether or chloroform and by heating at 60°C for 10 minutes. It is stable between pH 5 and 10 and antigenic variations have not been demonstrated. A variety of tissue culture systems from different animal species support viral replication; the most commonly used are those of porcine origin. There is no definite evidence that HC virus produces cytopathic effects in any of the cell systems so far tested[3] and replication must therefore be assessed by indirect methods such as the fluorescent antibody technique. The virus is still unclassified but, judging from its morphological, chemical, physical and serological characteristics, it is related to the virus of bovine diarrhoea. It has been suggested that these two viruses should be included in the Toga group together with the encephaloarboviruses [13].

On the other hand, ASF is a large DNA virus with a diameter of approximately 200 nm and at least 812 capsomeres. Its infectivity is destroyed by treatment

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with ether and chloroform and by heating at 60°C for 20 minutes. It is stable within a broad range of pH values. It replictates rapidly in cultures of blood or bone marrow leukocytes originating from swine and in the presence of red blood cells of the same animal species it usually shows a typical haemdasorbing effect. The cultivation of the virus in primary tissue cultures or in cell lines is difficult and a period of adaptation is required before cytopathic effects can be observed [9]. The virus of ASF elicited the production of complement fixing, precipitating and fluorescent antibodies but neutralizing antibodies were not detected. From the results of immunity tests in pigs it appears that more than one immunological type exists. ASF virus has been provisionally included in the Irido group[1].

*The Role of Wild Pigs in the Epizootiology of ASF*

It has been demonstrated that, in Africa, warthogs *(Phaecchoerus aethiopicus)*, bushpings *(Potamochoerus porcus)* and giant forest hogs *(Hylochoerus meinertzhageni)* may act as reservoirs of the virus of ASF[9]. The virus has also been isolated from a hippopotamus[7], porcupine and hyaena but these latter isolations have not been repeated subsquently [5 26]. If the disease becomes established in the domestic pig population the number of animals that may survive the infection increases and they may act as virus carriers for the rest of their lives. In South Africa, and in other countries where stringent control measures have been implemented since the first appearance of the disease, it appears that the virus has on the whole, been successfully contained in the wild population and that the domestic pig is not playing a carrier role in the epizootiology of the disease.

From a survey of the geographical distribution of warthogs carried out by the I.B.A.H.[2], it appears that ASF occurs in domestic pigs only where the presence of the wild species has been recorded. On the other hand, some areas inhabitated by warthogs appear to be free from the disease, suggesting that not all warthog populations are reservoirs of the virus. This situation prevails in South Africa, where warthogs are still present in large numbers in the Transvaal and Zululand (Natal); in other parts of the country they either do not exist or merely survive in small numbers. It has been demonstrated on several occasions that the warthogs in the northern, eastern and western Transvaal are infected with the virus of ASF [2 5 6] and the disease occurs sporadically in domestic pigs. In the Cape Province the disease has occurred in the past[15] but apparently the infection was never established in any wild animal species. In Zululand the disease has never been observed and all attempts to isolate the virus from wild animals have failed[2 25 28]. These observations were confirmed more recently by Keep (personal communication), who attempted to isolate the virus by inoculating domestic pigs with suspensions of lymph node and spleen obtained from adult and young warthogs approximately 10 months old. In addition sera obtained from warthogs living in the same area gave negative results when examined by the agar gell precipitation test for the detection of antibody (Pini&Keep, unpublished observations).

*The Transmission of the Virus of ASF*

When warthogs were infected with the virus of ASF they did not show clinical symptoms of the disease, although the virus could be isolated from these apparently healthy animals[7]. Whether warthogs, once infected, remain carriers of the virus for life has not been established. De Tray[7] was able to isolate the virus from infected animals for at least 54 days post inoculation. Plowright, Parker & Peirce[18] suggested that to determine the carrier status of warthogs, animals in the 4 to 12 month age group should be tested; they pointed out that the chances of virus isolation decrease as the animals grow older. This may support the hypothesis that warthogs are temporary carriers of the virus. These authors also found that it was extremely difficult to demonstrate viraemia in carrier warthogs. The spleen was a poor source of virus; lymph nodes were the most rewarding tissues, the highest concentration of virus in them being in the order of $10^5$ infective doses per g of infected tissue. This, however, is a low level when compared with the concentration of virus detectable in the tissues of domestic pigs undergoing the acute course of the disease.

Although it has been proved beyond doubt that warthogs play a role in the epizootiology of ASF it is not yet clear how the infection is transmitted from wild pig to wild pig or from wild to domestic pig. Montgomery[14] gave evidence that ASF is not an airborne infection, since susceptible domestic pigs remain healthy if they were merely separated by a wire gauze fence from infected ones excreting high concentrations of virus. Rooting, eating and drinking in infected premises appeared to be essential for acquisition of the infection.

The virus of ASF was never detected in the secreta or excreta of warthogs and all attempts to infect either domestic pigs or susceptible wild pigs by housing them with carrier warthogs failed even when they lived together for long periods of time[7], (Plowright & Parker, unpublished observations, 1967). Because the disease appears to have a seasonal incidence coinciding with the farrowing time of the wild pigs, it was suggested that virus reactivation could occur at this time of the year[22 23]. The limited data available[18] do not seem to support this theory but this aspect requires confirmation. In western Uganda young warthogs under 3 months old were found to be free from infection and virus was not isolated from foetal material or from mammary tissues of lactating carrier warthogs. Plowright, Parker & Pierce[18], investigating ways of transferring the virus, found that 10 infective doses given by the parenteral route consistently infected domestic pigs. On the other hand they failed to infect domestic pigs by feeding them with homogenized lymph node tissue suspensions containing up to $10^5$ infective doses of virus. They concluded that under natural conditions it was unlikely that domestic pigs could become infected by the ingestion of warthog offal, which does not contain such high concentrations of virus. They suggested that the tick *O. moubata* was the missing link for the transmission of ASF virus among wild pigs and from warthogs to domestic pigs.

*The Role of the Argasid Tick (O. moubata) in the Epizootiology of ASF*

Montgomery[14] and Walker[29] were among the first to suggest that the link for transmission of ASF between warthogs and domestic pigs could be a blood-sucking

insect. In subsequent years various investigators[7 10 11] failed to isolate ASF virus from *Rhipicephalus* and *Ornithodoros* ticks collected from carrier warthogs and burrows. However, in 1963 Botija[4] succeeded in isolating the virus in Spain from specimens of *O. erraticus* that had previously fed on infected domestic pigs. This finding was extended by Plowright, Parker & Pierce [17], who succeeded in isolating the virus from *O. moubata* collected from warthog burrows in East Africa.

From the report by Theiler[27] it appears that *O. moubata* has been recorded in most African countries. In South Africa it is widely distributed in the northern, eastern and western Transvaal and along the coastal strip of Zululand and has also been found in the Cape Province. The Orange Free State appears to be free. The immature and adult ticks are parasites of lizards (*Varanus* species) and other reptiles, anteaters (*Manis temnincki*), antbears (*Orycteropus afer*), warthogs (*P. aethiopicus*), bushpigs (*P. porcus*) and other mammals. The tick can also be found in human habitations and in burrows used by porcupines, warthogs and antbears.

Plowright *et al.*[17 18 19 20] in their investigations to establish the role played by *O. moubata* in the epizootiology of the disease, found that warthog burrows in Kenya and Tanzania were heavily infested with ticks, of which about 40% were infected with the virus of ASF. Under natural conditions all stages apart from the first, which does not feed, were found to harbour the virus. Frequency of infection increased with the age of the ticks and virus titres of $10^7$ infective doses could be obtained.

It is interesting to note that several thousand ticks collected from burrows in the Queen Elizabeth National Park in Uganda did not yield virus even though the infection is well established in the local warthog population.

In all instances the agent isolated from the ticks had the typical characteristics of ASF virus and, if a tick that had engorged on a reacting pig was transferred to a normal animal, the first symtoms of the disease could be seen after a mean incubation time of 6 days.

Under experimental conditions transovarial transmission was demonstrated and this was considered to be one of the mechanisms for the natural maintenance of the virus[19]. However, not all strains of ASF virus appeared to be equally effective in establishing a persistent infection in the tampans. Some strains could persistently infect 75% of the ticks whereas others could only infect 5%.[20] Greig[8] subsequently recorded the highest concentrations of virus, approximately $10^6$ infective doses, in the gut 100 days after infection whereas the coxal fluid and the saliva had lower concentrations[8].

The amount of virus required to infect ticks varied between $10^2$ and $10^5$ infective doses according to the strain under investigation[20]. From these results it appeared that to infect ticks under natural conditions the viraemic titres in the carrier warthogs had to be in the range of $10^3$ to $10^4$ infective doses per ml of blood and, according to these authors, these values have never been detected in any warthogs tested irrespective of age. They concluded that either another vertebrate host with high viraemic titres is involved in the epizootiology of the disease or that at certain stages in the life of warthogs, as yet undetermined, high levels of viraemia could be present.

*Hog Cholera in South Africa*

There is reason to believe that in South Africa the viruses of both ASF and HC were present at the same time, but in different areas of the country between the end of the last and the beginning of this century.

In 1903 an outbreak of a disease defined as swine fever was recorded at the government farm, Groot Constantia[12 21], and later in the Paarl District, of the Cape Province. According to Hutcheon[12] the same disease must have been active in 1900 on the Cape Flats.

In 1905 two outbreaks of swine fever occurred in the Malmesbury District of the western Cape and later, between 1910 and 1917, 13 more outbreaks were recorded in this area. By 1918 the infection had spread to the eastern Cape as far as Somerset East. During 1903 and 1904 the Transvaal was also sporadically affected with outbreaks in the Krugersdorp and Pretoria Districts. The veterinary authorities introduced strict and what appear to have been appropriate control measures. Infected pigs were slaughtered and buried, manure was destroyed and affected farms were prohibited from restocking for a period of 6 months. It was also appreciated that the outbreaks in the Transvaal were connected with movements of pigs from the infected areas in the Cape Province. To arrest further dispersion of the virus towards the northern parts of the country, Theiler in 1905 prohibited movements of pigs from the Cape to the Transvaal.

On the basis of the epizootiological observations and the description of certain pathological lesions, it seems likely that the disease occurring in the Cape Province between 1900 and 1918 was HC. During that period wild pigs were never associated with cases of swine fever and the outbreaks were always related to the movement of infected domestic pigs. In addition, the presence of reservoirs of ASF virus has never been demonstrated in the Cape Province. Frequent references to the presence of necrotic buttons in the intestines of the infected animals also appears to confirm the diagnosis of HC.

The disease was probably introduced into South Africa from England or other European countries that, as stated above, became infected with the virus between 1862 and 1887. The stringent control measures implemented were successful in limiting the spread of the virus; it did not become established in the domestic pigs nor in any other reservoirs. Consequently what is thought to have been HC was eradicated from South Africa by 1918.

*African Swine Fever in South Africa from 1926 to 1972*

Between 1918 and 1926 no cases of swine fever were recorded in this country.

In 1921 Montgomery[14] described for the first time what he called East African swine fever and stated that wild pigs were the source of the infection. In 1926 several outbreaks of a serious disease were recorded in the Potgietersrus District in northern Transvaal. This disease was observed more often in free-living domestic pigs, when warthogs were present on the farm, and its similarity with the disease occurring in Kenya was noticed.

The control measures applied were similar to those implemented earlier in the Cape Province. They con-

sisted of slaughtering both the affected animals and the survivors; the destruction of manure; the prohibition from restocking farms within a period of 3 months, and control of the movements of pigs and pig products. The implementation of these measures was hampered by the distances between farms, poor hygienic conditions and difficulties in keeping animals in sties to avoid contact with the wild pigs.

Between 1935 and 1938, 10 outbreaks of the disease were recorded in the Soutpansberg, Pietersburg, Potgietersrus and Waterberg Districts of northern Transvaal. In some cases the origin of the infection was obscure, in others it was associated with the presence of wild pigs or with the feeding of animals with swill from infected farms. ASF also occurred in 1934 in the Witwatersrand area but the source of infection remained unknown.

In 1933 the disease broke out, it is believed for the first time, in the Cape Province among pigs for slaughter at the Imperial Cold Storage, Gouda, Tulbagh District. The origin of the infection was apparently traced to a consignment of animals from Johannesburg. A second outbreak occurred after a short interval on a farm in the Wellington District and again a consignment of pigs from the Transvaal was considered to be the source of the infection. De Kock [6] stated that these two outbreaks were responsible for the enzootic that affected the Cape in the following years. In early 1934, ASF occurred in the outskrits of Cape Town, Malmesbury, Moorreesburg, Piketberg, Clanwilliam, Wellington and Paarl. Quarantine was proclaimed in an area of 600 square miles. In 1936 the disease re-appeared and 5 outbreaks were confirmed in Caledon, Wellington, Franshoek and Worcester. In these instances the infection was local in origin[6]. In a final attempt to eradicate the disease as many pigs as possible were sent to the abattoir and buffer farms, without pigs or with pigs in sties, were created around the infected areas.

The last recorded outbreak in the Cape occurred in Piketberg District in 1939 after an interval of 4 years from the previous outbreak. The origin of the infection was traced to a neighbouring farm where mortality due to the virus of ASF was reported to occur annually in October.

It may be concluded that the control measures implemented resulted in the eradication of ASF in the Cape Province by 1939. The infection had entered the area through the movement of domestic pigs from the Transvaal and the disease did not become established in the local domestic pig population. All animals that could have been chronically infected were eventually destroyed. In addition the virus did not manage to escape from the domestic pigs and establish itself in any other reservoir. This was probably also due to the ecological situation in the Cape, where wild pigs were not as numerous as they were in the Transvaal.

Swine fever was not recorded in South Africa between 1939 and 1951. In 1951 the disease appeared again in the Transvaal: 3 outbreaks were recorded in the districts of Pietersburg, Soutpansberg and Letaba and another near Pretoria. The areas of Roodepoort, Krugersdorp and Boksburg were also affected and in the latter case the origin of the infection was traced to a consignment of infected animals from S.W.A. Between 1953 and 1962, 17 outbreaks were confirmed in the northern and eastern Transvaal. In 1962 ASF apparently vanished and no cases were reported until May 1973.

It is interesting to observe that in domestic pigs in South Africa ASF is apparently manifest cyclically, with 4 cycles occurring between 1926 and 1972. Two of these cycles were of active disease, the first lasting 12 years from 1926 to 1938 and the second 11 years from 1951 to 1962. In between there were 2 cycles in which the virus was silent, the first lasting 11 years from 1939 to 1950 and the 2nd 10 years from 1963 to 1972. It appears that in June 1973 the 3rd active cycle started.

*ASF in South Africa in 1973 and 1974*

Between May 1973 and March 1974, 18 outbreaks of ASF were recorded and confirmed by isolation of the virus. They all occurred in the controlled area in non-approved piggeries that are allowed to keep pigs only for local consumption.

These 18 outbreaks can apparently be grouped into 6 primary foci of infection. The first was reported on May 29th, 1973 in the eastern part of Letaba District on a farm called Lillie. The origin of the infection was attributed to a warthog that was found and killed on the farm and the meat used for human consumption. From all the specimens obtained on this farm from different animals at different time intervals a non-haemadsorbing strain of ASF virus was isolated[16]. The second case of disease occurred a month later on another farm in the same district approximately 35 km from the previous outbreak. The origin of this infection was not established. The virus isolated was a typical haemadsorbing strain of ASF and it was assumed that this later outbreak was a new primary focus of infection.

A 3rd focus was recorded in Pietersburg District. The source of the infection was not traced. Three of the 4 piggeries affected were on adjoining farms in Lebowa and the 4th piggery was approximately 60 km away. It was found that on the latter farm pigs were fed on meat scraps obtained from a local butcher who had probably purchased meat from one of the farms previously affected.

The 4th focus of infection occurred in the Gazankulu homeland and the adjoining farms in the western part of Letaba District. Between the end of September and the end of November 7 outbreaks were confirmed. They probably all had a common origin and the disease was spread through the movement of affected pig products.

The 5th focus of disease was recorded in White River District between December 1973 and January 1974. All the farms affected adjoin and wild pigs are found in the area.

At the time when this report was written, a further focus of infection was confirmed on a farm in the Thabazimbi District in the western Transvaal. This area had recently been surveyed and it had been found that *O. moubata* infected with the virus of ASF was present in the burrows used by the warthogs.

During the period May 1973 and March 1974 between 3 500 and 4 000 pigs died of the disease or were destroyed because of contact with infected animals.

It may be concluded that the results of the investigations to establish the primary sources of infection during the recent outbreaks of ASF were not entirely satisfactory. Some of the areas affected are obviously inhabited by wild pigs and they must have played a role as source of infection.

The primary causes of the sudden flare-up of the disease are also obscure. With most infectious diseases those animals that have been exposed to the agent concerned become immune to it. Further outbreaks only occur when a susceptible population has again developed. In the case of ASF the domestic pig population is always fully susceptible to the virus. However, no data are available in this respect concerning wild pigs.

The present situation appears to confirm the statement made by De Tray[7] that: "ASF is one of the most complex problems in the history of infectious diseases. Much remains to be explained."

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REVIEW OORSIG

# TRICHINOSIS (*Trichinella spiralis* INFESTATIONS) IN WILD ANIMALS OF THE KRUGER NATIONAL PARK

E. YOUNG AND I.J. WHYTE.*

SUMMARY

In Africa trichinosis is essentially a disease of wild carnivores. Once established in a suilline cycle it becomes a more important threat to man. The results of tests on 8 000 specimens of 20 wildlife species in the Kruger National Park are discussed and the epizootiology of trichinosis in South Africa is briefly reviewed. The first case of trichinosis in an African civet, *Viverra civetta* Schreber, 1776, is reported. The confirmed absence of trichinosis in true herbivores is of practical significance in view of the increasing utilization of game meat as food.

In Europe and America man usually becomes infested with *T. spiralis* when eating the flesh of domestic pigs which have been fed on infective pork products or offal[1]. In these parts of the world human trichinosis may also result from the ingestion of infective products of wild animal origin. The bush-pig, *Potamochoerus porcus*, seems to have been the only source of human infestation in East Africa[1]. Human trichinosis has, to the best of our knowledge, not yet been recorded in South Africa.

Until 1966 South Africa had been considered to be free of trichinosis. In this year, however, *T. spiralis* was found in a paralysed lion, *Panthera leo*, near the Lower Sabie tourist camp in the Kruger National Park, South Africa[3]. A subsequent and very intensive countrywide survey of domestic pigs proved that, with the exception of the Kruger Park, South Africa is still free of trichinosis (P.R. Mansvelt, *pers. comm.*). Examination of 8 000 specimens from 20 wildlife species in the Kruger Park have indicated that *T. spiralis* only occurs in the southern part. This survey is still continuing.

In addition to lion, trichinosis has since 1966 also been diagnosed in 5 other mammal species in the Kruger National Park, including spotted hyena, *Crocuta crocuta*, black-backed jackal, *Canis mesomelas*, and the multimammate mouse, *Praomys (Mastomys) natalenses*[5]. The name of the African civet can now also be added to the list of naturally infested species. It was found that *T. spiralis* may be responsible for paralysis in this nocturnal, cat-like viverrid. The finding of *T. spiralis* in a warthog, *Phacochoerus aethiopicus*[2] is of more practical importance as the meat of this species is considered to be a very special delicacy by many inhabitants of this continent.

In free living wildlife populations, the host range of *T. spiralis* seems to be restricted to predatory and scavenging species. This observations is hardly surprising as the ingestion of infested meat is the only important mode of transmission.

Hyenas have been incriminated as the most important natural host [1 3 5]. Spotted hyenas are known to occasionally kill and devour especially old and sick lions, members of its own species, other carnivores and rodents. Natural infestations can therefore be easily maintained in this species. The infestation rates in hyenas of 44% in parts of East Africa[1] and about 85% in the Skukuza region of the Kruger Park[5] is thus also not surprising.

There is usually an increased tendency to canabalism in relative overpopulations of wild carnivores and rodents. The opportunities for natural transmission of trichinosis are extremely favourable under such circumstances. Depending on the lethal effects of trichinosis on wild animals, it may play an important rôle in the natural regulation of population numbers of wild carnivores and rodents[5].

While still alive, infested wild carnivores can hardly transmit trichinosis to man or his domestic animals. Hyenas, lions and other wild predators and scavengers are, however, shot on many farms, adjoining game reserves. Carcasses are seldom destroyed but are usually taken to the homesteads for removal of trophies. On these farms, infested meat and organs may serve as important sources of infestation to dogs, cats, pigs and some other domestic animals. Infested pigs may then initiate outbreaks of human trichinosis. Africans of certain tribes, who do sometimes partake of lion meat, may become infested in this way. Witch-doctors may also aid in the propagation of human trichinosis. In some parts of Africa they ascribe certain mysterious powers to the hyena and prescribe certain parts of hyenas' bodies in the 'treatment' of their patients.

The Kenyan strain of *T. spiralis* is known to be of lower infectivity to domestic pigs than the European stain[1]. Pigs have also been proved to be considerably less suspectible to infestation with the Kruger Park strain[2] and it is suspected that this strain of *T. spiralis* will probably not establish itself in a suilline cycle. Every precaution should, however, be taken to prevent the spread of trichinosis from the known relatively small infested wildlife focus to man and his domestic animals elsewhere in the country. In this

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regard, the transport of known susceptible and possibly infested species, their carcasses or their products from known infested to clean areas should especially be very strictly controlled.

In parts of the world where rats or other rodents are highly susceptible to the local strains of *T. spiralis*, especially in urban areas in Europe and America, they may play an important role in the maintenance of a domestic transmission-cycle which can be of major public health significance[1]. Although wild carnivores are apparently playing a more important role in the epizootiology of trichinosis in Africa, the occurrence of trichinosis in wild rodents in the Kruger Park[5] should also be viewed with concern. Almost every carnivorous mammal and bird species preys on rodents and the latter may, therefore, serve as suitable sources of infestation to a great variety of other species. Infested rodents may also be accidentally transported over long distances with veld hay, other farm produce, furniture, etc., thereby establishing new infestations in areas, previously free of trichinosis. Domestic cats and even dogs from infested areas, which could have become infested by feeding on infested rats, mice or infested animal products may also contribute to the regional distribution of this parasite when transported to clean areas.

Active steps are taken to restrict the distribution of *T. spiralis* in South Africa and research projects are in progress to obtain further information on the pathogenesis and epizootiology of trichinosis in susceptible wildlife species. Experimental treatment of infested wild animals is also receiving attention in order to find practical and satisfactory ways of ensuring the transport of only parasite free individuals from infested to clean areas.

Muscle specimens of the following wildlife species in the Kruger National Park have been examined with negative results. (Numbers of animals are given in brackets) : Cheetah, *Acinonyx jubatus (5);* leopard, *Panthera pardus* (1); genet, *Genetata* spp. (3) ; banded mongoose, *Mungos mungo* (2) ; porcupine, *Hystrix africae-australis* (1) ; Chacma baboon, *Papio ursinus* (4) ; Cape buffalo, *Syncerus caffer* (2 223) ; giraffe, *Giraffa camelopardalis* (1) ; blue wildebeest, *Connochaetes taurinus* (1 146) ; kudu, *Tragelaphus strepsiceros* (1) ; reedbuck *Redunca aruwdinum* (1); impala, *Aepyceros melampus* (2 093) ; Burchell's zebra, *Equus burchelli* (310); African elephant, *Loxodonta africana* (2 030), and Cape vulture, *Gyps coprotheres* (1).

As Africa's wildlife is already providing a considerable proportion of the animal protein consumed by its human inhabitants from 60% to over 80% in certain countries[4] - the absence of trichinosis in such a large sample of meat producing wild herbivores is rather relieving. This is particularly true in the case of elephant and buffalo, of which considerable numbers have to be culled in the Kruger National Park. Zebra, blue wildebeest, impala and other African antelope species are being utilized to an increasing extent for game farming and meat production in Southern Africa and, from a public health point of view, the above findings are also of considerable practical significance with regards to these species.

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## New Liver-Biopsy Technique

In residue studies at the Veterinary Toxicology and Entomology Research Laboratory (College Station, Texas), veterinarians are using new surgical procedures for taking large liver-samples. These procedures permit samples ranging from 25 to 100 grams to be taken, with relative safety, from live animals.

In view of high mortality rates, due to excessive bleeding as a result of the numerious blood vessels having been damaged and the extremely soft nature of the tissue, the removal of large samples is normally kept to a minimum. Consequently, in most cases, very small amounts of tissue are removed, using a hollow needle.

To circumvent the haemorrhaging which inevitably occurs when taking large samples, a row of vertical mattress sutures is placed along the incision line before the liver is incised. Deviating from standard practice, however, the surface area of the suture material is increased. Small pieces of plastic tubing are threaded on to the sutures during stitching, so that the tubing is on both the upper and lower surfaces of the liver, to act as compression pads. The sutures can then be drawn tighter than normal to produce the necessary pressures to stop bleeding. The new method therefore enables the surgeon to immediately proceed with the operation, without waiting to check whether or not the bleeding has stopped.

Apart from the obvious value which this technique has in the veterinary medical field, it may also find application in human medicine, e.g. it could possible be used to stop bleeding caused by liver damage incurred by persons involved in serious accidents.

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# THE EFFECT OF PHENCYCLIDINE ANAESTHESIA ON THE BLOOD CHEMISTRY AND HAEMATOLOGY OF THE CHACMA BABOON *(PAPIO URSINUS)*

D.G. STEYN*


## SUMMARY

Blood was collected from 14 baboons 3 - 5 min, 30 min and 60 min after the intramuscular injection of phencyclidine at a dosage rate of 1,5 mg/kg body mass. The determination of blood chemistry and haematology was then undertaken. An abrupt decrease in total plasma protein occurred after the injection of phencyclidine but thereafter it remained at approximately the same level. The sodium, potassium, chloride and cholesterol remained practically unaltered throughout. A significant decrease occurred in the blood urea level. The blood sugar value showed a highly significant decrease over the first 30 min period and less over the second 30 min period. Plasma enzyme activity and corticosteroid levels did not show marked alterations.

Marked and significantly lowered values occurred in white blood cell count, erythrocyte count, haemoglobin concentration and haematocrit level but these values returned to within normal limits at 60 min.


## INTRODUCTION

Baboons are very powerful animals and they are capable of inflicting severe damage with their razor sharp canine teeth. To safeguard personnel against injury, all baboons are routinely anaesthetized to facilitate specimen collection and any other experimental procedures. Phencyclidine (Sernylan - Parke Davis and Co.) is at present the drug of choice for the immobilization of baboons. The compound is given by the intramuscular route which makes its administration very convenient. Moreover, phencyclidine has been used with good results as a sedative and for the induction of anaesthesia at this particular primate colony. In addition, it is possible to maintain an animal in a state of light anaesthesia by the repetitive administration of the drug at required intervals.

Since anaesthesia is required for specimen collection, the animal's normal state may well be altered by the anaesthetic agent. Rümke[6] stated that the effect of anaesthetics is not restricted to cells of the central nervous system but nearly every cell in the body may be affected in its function. He suggested that most of the effects of general anaesthetics on organs or organ systems are, however, only temporary.

Under colony conditions, specimens are normally collected about 30 min after the administration of the anaesthetic agent. Thus, the purpose of this investigation was to explore the possible role played by anaesthesia in producing changes in the blood chemical and haematological values over the first hour following the administration of phencyclidine.

## MATERIALS AND METHODS

In order to determine the effect of this procedure on the blood chemistry and haematology, 14 conditioned baboons were injected with this agent. Of the 14 baboons six were males and eight were females. Their masses ranged from 10 to 19 kg.

After the intramuscular injection of the phencyclidine, time was allowed for the baboons to become sufficiently sedated to avoid any risk to the handler. This usually required 3-5 minutes after the administration of 1,5 mg/kg of phencyclidine. Specimens were collected at 3-5 min following the injection and again at 30 min and 60 min. The procedures for the collection of blood and the methods employed for blood chemical and haematological determinations have been described previously[8].

## RESULTS

### 1. BLOOD CHEMISTRY

The blood chemistry changes are presented in table 1. Values for male and female animals are tabulated separately in table 2 for those parameters in which statistically significant changes could be demonstrated.

#### *a. Plasma proteins*

The total plasma proteins (TPP) had an initial value which was higher than the mean value determined for conditioned baboons. The value decreased markedly from the initial value to the level found at 30 min after the injection of phencyclidine. There was very little difference between the concentration found at 30 min and the value measured at 60 min. It was noted that the decrease in the males was not significant whereas the female levels were significantly less ($P < 0,05$). Significantly lower values ($P < 0,05$) also occurred in the females with regard to albumin and alpha-2 globulin. Changes in the males were similar but not significant.

#### *b. Electrolytes*

The mean potassium value in the females decreased considerably from 3-5 min to the 30 min recording

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TABLE 1: THE EFFECT OF PHENCYCLIDINE ANAESTHESIA ON THE BLOOD CHEMISTRY OF THE CHACMA BABOON

| Determination | N | 3 – 5 min | 30 min | 60 min | SL 1 – 2 | SL 1 – 3 | SL 2 – 3 |
|---|---|---|---|---|---|---|---|
| Plasma Protein | 14 | 7,16 ± 0,76 | 6,86 ± 0,76 | 6,72 ± 0,47 | – | P<0,05 | – |
| Albumin | 14 | 3,48 ± 0,73 | 3,31 ± 0,69 | 3,23 ± 0,58 | – | P<0,05 | – |
| Alpha-1 globulin | 14 | 0,17 ± 0,04 | 0,17 ± 0,04 | 0,18 ± 0,04 | – | – | – |
| Alpha-2 globulin | 14 | 0,67 ± 0,11 | 0,64 ± 0,09 | 0,61 ± 0,09 | – | P<0,05 | – |
| Beta globulin | 14 | 1,35 ± 0,21 | 1,27 ± 0,20 | 1,26 ± 0,13 | – | P<0,05 | – |
| Gamma globulin | 14 | 1,50 ± 0,33 | 1,45 ± 0,31 | 1,43 ± 0,36 | – | – | – |
| Potassium | 14 | 4,15 ± 0,51 | 3,86 ± 0,60 | 4,07 ± 0,63 | – | – | – |
| Sodium | 14 | 143,00 ± 7,12 | 145,00 ± 2,42 | 144,00 ± 3,06 | – | – | – |
| Chloride | 14 | 104,00 ± 6,07 | 104,00 ± 5,48 | 106,00 ± 4,40 | – | – | – |
| $CO_2$ Content | 11 | 23,54 ± 5,72 | 28,07 ± 3,30 | 29,55 ± 2,13 | P<0,01 | P<0,01 | – |
| Urea | 14 | 41,00 ± 6,60 | 38,00 ± 7,73 | 39,00 ± 6,70 | P<0,01 | P<0,01 | – |
| Cholesterol | 14 | 133,00 ± 35,51 | 126,00 ± 31,86 | 140,00 ± 42,58 | – | – | – |
| Calcium | 14 | 5,09 ± 0,35 | 5,04 ± 0,30 | 5,01 ± 0,29 | – | – | – |
| Phosphorus | 14 | 1,34 ± 0,61 | 1,27 ± 0,66 | 1,26 ± 0,55 | – | – | – |
| Glucose | 14 | 159,00 ± 47,44 | 99,00 ± 29,75 | 89,00 ± 21,65 | P<0,01 | P<0,01 | – |
| SAP | 14 | 735,00 ± 406,00 | 679,00 ± 380,00 | 725,00 ± 399,00 | – | – | – |
| SGPT | 14 | 12,00 ± 5,81 | 12,00 ± 4,99 | 11,00 ± 3,09 | – | – | – |
| SGOT | 14 | 13,00 ± 3,02 | 14,00 ± 2,67 | 13,00 ± 2,03 | – | – | – |
| LDH | 14 | 226,00 ± 47,90 | 272,00 ± 163,00 | 243,00 ± 96,33 | – | – | – |
| Corticosteroids | 14 | 23,40 ± 10,21 | 21,90 ± 11,04 | 23,60 ± 12,49 | – | – | – |

N = number of samples; 1–2 = statistical significance levels of differences between 3 – 5 and 30 min sampling; 1 – 3 = statistical significance levels of differences between 3 – 5 and 60 min sampling; 2 – 3 = statistical significance levels of differences between 30 min and 60 min sampling; SAP = serum alkaline phosphatase; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase; LDH = lactic dehydrogenase; ± = standard deviation.

TABLE 2: THE EFFECT OF PHENCYCLIDINE ANAESTHESIA ON THE BLOOD CHEMISTRY OF THE CHACMA BABOON – DIFFERENCE IN RESPONSE BETWEEN MALES AND FEMALES

| Determination | Sex | N | 3 – 5 min | 30 min | 60 min |
|---|---|---|---|---|---|
| Plasma Protein | M | 6 | 7,42 ± 0,52 | 7,02 ± 0,63 | 6,85 ± 0,33 |
| | F | 8 | 6,97 ± 0,89 | 6,74 ± 0,87 | 6,62 ± 0,56 |
| Albumin | M | 6 | 3,88 ± 0,56 | 3,68 ± 0,57 | 3,60 ± 0,41 |
| | F | 8 | 3,18 ± 0,74 | 3,03 ± 0,67 | 2,96 ± 0,56 |
| Alpha-2 globulin | M | 6 | 0,63 ± 0,09 | 0,63 ± 0,08 | 0,57 ± 0,08 |
| | F | 8 | 0,69 ± 0,12 | 0,65 ± 0,11 | 0,65 ± 0,09 |
| A/G ratio | M | 6 | 1,09 ± | 1,10 ± | 1,11 ± |
| | F | 8 | 0,84 ± | 0,80 ± | 0,80 ± |
| $CO_2$ Content | M | 6 | 22,47 ± 8,25 | 26,75 ± 5,10 | 30,93 ± 1,73 |
| | F | 8 | 24,14 ± 4,39 | 28,83 ± 1,80 | 28,76 ± 2,01 |
| Urea | M | 6 | 42,83 ± 7,44 | 39,17 ± 8,84 | 39,50 ± 5,86 |
| | F | 8 | 40,00 ± 6,12 | 37,00 ± 7,27 | 38,13 ± 7,61 |
| Calcium | M | 6 | 5,13 ± 0,41 | 4,90 ± 0,21 | 5,05 ± 0,32 |
| | F | 8 | 5,06 ± 0,33 | 5,14 ± 0,32 | 4,97 ± 0,28 |
| Blood sugar | M | 6 | 146,33 ± 57,35 | 89,83 ± 36,92 | 82,83 ± 27,13 |
| | F | 8 | 168,00 ± 40,01 | 105,00 ± 23,61 | 92,75 ± 17,22 |
| SGPT | M | 6 | 10,83 ± 3,49 | 10,00 ± 1,10 | 10,50 ± 2,07 |
| | F | 8 | 13,63 ± 7,09 | 13,75 ± 6,20 | 11,00 ± 3,82 |

N = number of samples; A/G ratio = Albumin/globulin ratio; SGPT = serum glutamic pyruvic transaminase; ± standard deviation; M = male; F = female.

whereas the sodium value increased over the same period. The male values remained unaltered. Chloride concentrations did not show any notable deviations. The carbon dioxide content increased significantly ($P<0,01$) during the first 30 min of anaesthesia. The value showed a further increase over the following 30 min although this increase was not significant. The elevation was, however, highly significant ($P<0,01$) when the value was compared with the initial value.

*c. Blood urea*

A significant ($P < 0,01$) decrease occurred in the blood urea during the first 30 min period. Over the interval between 30 min and 60 min it increased slightly

but the difference between the first and 60 min mean values was still significant ($P < 0,01$).

*d. Total cholesterol*

The cholesterol values did not show any notable changes during the experiment.

*e. Calcium and inorganic phosphorus*

The calcium value increased significantly ($P < 0,5$) in males from the 30 min sampling to the 60 min sampling after an initial insignificant increase between the 3-5 min and 30 min determinations. However, when the value for both sexes was examined, no significant changes were found.

*f. Blood sugar*

The blood sugar manifested the most dramatic changes of all the parameters determined. The value obtained from the first specimen was significantly ($P < 0,01$) higher than the value obtained for normal baboons adapted to the conditions in this primate colony. This very high initial value decreased significantly ($P < 0,01$) over the following 30 min period. A further decrease occurred over the second 30 min period.

When the results obtained for the two sexes were compared, it was found that considerably higher blood sugar values were recorded at the first sampling in the females than in the males. The 30 min and 60 min values for females were also higher than for the males although to a lesser degree than the initial value. The higher blood sugar levels found in the females seemed to confirm the sex related differences in blood sugar values which was evident in standard values[8].

*g. Serum enzymes*

A significant ($P < 0,05$) decrease occurred in the serum glutamic pyruvic transaminase (SGPT) values in the females between the 30 min and 60 min determinations. This decrease reflected the only significant alteration in the activity of the enzymes studied. The alkaline phosphatase was consistently higher in the males than in the females. This was also demonstrated in the standard values for conditioned baboons.

TABLE 3: THE EFFECT OF PHENCYCLIDINE ANAESTHESIA ON THE HAEMATOLOGY OF THE CHACMA BABOON

| Determination | N | 1<br>3 – 5 min | 2<br>30 min | 3<br>60 min | 1 – 2 | 1 – 3 | 2 – 3 |
|---|---|---|---|---|---|---|---|
| White blood cell count | 14 | 9529,00 ± 3433,00 | 7443,00 ± 2533,00 | 7800,00 ± 2468,00 | $P<0,01$ | $P<0,01$ | – |
| Lymphocytes | 14 | 40,57 ± 11,73 | 39,57 ± 9,64 | 39,00 ± 9,66 | – | – | – |
| Neutrophils | 14 | 55,14 ± 10,81 | 59,14 ± 9,51 | 57,57 ± 10,06 | – | – | – |
| Monocytes | 14 | 2,00 ± 1,41 | 0,86 ± 0,90 | 1,57 ± 0,79 | – | – | – |
| Eosinophils | 14 | 2,00 ± 2,89 | 0,29 ± 0,76 | 1,71 ± 1,38 | – | – | – |
| Basophils | 14 | 0,29 ± 0,49 | 0,14 ± 0,38 | 0,14 ± 0,38 | – | – | – |
| Red blood cell count | 14 | 5,64 ± 0,40 | 5,52 ± 0,31 | 5,63 ± 0,35 | – | – | – |
| Haemoglobin | 14 | 14,56 ± 0,83 | 14,14 ± 0,57 | 14,45 ± 0,53 | $P<0,01$ | – | $P<0,05$ |
| Haematocrit | 14 | 45,29 ± 3,29 | 43,86 ± 2,48 | 44,79 ± 2,91 | $P<0,01$ | – | – |
| ESR | 14 | 3,36 ± 4,33 | 2,71 ± 2,46 | 2,86 ± 2,38 | – | – | – |
| MCV | 14 | 80,21 ± 3,12 | 79,86 ± 2,98 | 80,29 ± 3,12 | – | – | $P<0,05$ |
| MCH | 14 | 25,71 ± 1,20 | 25,57 ± 1,16 | 25,64 ± 1,01 | – | – | – |
| MCHC | 14 | 31,93 ± 1,00 | 31,86 ± 0,77 | 31,71 ± 1,33 | – | – | – |

N = number of samples; 1 – 2 = statistical significance levels of differences between 3 – 5 min and 30 min sampling; 1 – 3 = statistical significance levels of differences between 3 – 5 min and 60 min sampling; 2 – 3 = statistical significance levels of differences between 30 min and 60 min sampling; ± = standard deviation; ESR = erythroeyte sedimentation rate; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

TABLE 4: THE EFFECT OF PHENCYCLINDINE ANAESTHESIA ON THE HAEMATOLOGY OF THE CHACMA BABOON – DIFFERENCE IN RESPONSE BETWEEN MALES AND FEMALES.

| Determination | Sex | N | 3 – 5 min | 30 min | 60 min |
|---|---|---|---|---|---|
| White blood cell count | M | 6 | 8767,00 ± 5489,00 | 6820,00 ± 3276,00 | 7050,00 ± 3194,00 |
| | F | 8 | 10100,00 ± 2692,00 | 7912,00 ± 1913,00 | 8362,00 ± 1784,00 |
| Red blood cell count | M | 6 | 5,65 ± 0,32 | 5,53 ± 0,22 | 5,55 ± 0,21 |
| | F | 8 | 5,62 ± 0,47 | 5,52 ± 0,39 | 5,69 ± 0,43 |
| Haemoglobin | M | 6 | 14,92 ± 0,50 | 14,30 ± 0,22 | 14,47 ± 0,45 |
| | F | 8 | 14,29 ± 0,95 | 14,01 ± 0,73 | 14,44 ± 0,62 |
| Haematocrit | M | 6 | 46,17 ± 2,14 | 44,67 ± 1,37 | 44,67 ± 1,03 |
| | F | 8 | 44,62 ± 3,96 | 43,25 ± 3,01 | 44,87 ± 3,87 |

N = number of samples; M = male; F = female; ± = standard deviation.

*h. Plasma corticosteroids*

The concentration of plasma corticosteroids did not show any deviations from the normal values and no difference between male and female animals could be demonstrated.

2. HAEMATOLOGY

The results of the effect of phencyclidine anaesthesia on the haematological determinations are tabulated in table 3. Results obtained when the sexes were separated, are found in table 4.

*a. Leukocytes*

The white blood cell count decreased significantly ($P < 0,01$) from the initial to the 30 min sampling. The 60 min value was also significantly ($P < 0,01$) less than the initial value, but there was very little difference between the 30 min and 60 min values.
The differential leukocyte count showed very little change at the various sampling occasions. The very marked lymphopenia and eosinopenia with the concomitant neutrophilia usually encountered in severe stress or adrenocortical hyperfunction[2] were not seen in these baboons.

*b. Erythrocyte count, haemoglobin and haematocrit*

The erythrocyte count decreased slightly over the first 30 min period but returned to practically the same level as the initial value over the second 30 min period. The haemoglobin concentration decreased significantly ($P < 0,01$) over the first 30 min period, but increased significantly ($P < 0,01$) over the following 30 min to a value approximating the initial value. The changes in haematocrit followed the haemoglobin results rather closely, presenting a significant ($P < 0,01$) decrease over a level just below the initial value.

*c. Erythrocyte sedimentation rate and red cell indices*

The erythrocyte sedimentation rate and the red cell indices remained within normal limits except in the case of the MCV which increased over the second 30 min interval. This was more pronounced in the males than in the females.

DISCUSSION

The decrease in the TPP, albumin and some of the globulin fractions as well as urea was probably due to a shift of fluid from extravascular to intravascular compartments. However, the mechanism by which this shift of fluid is brought about, is not clear. Similar findings were recorded for the erythrocyte count, haemoglobin concentration and haematocrit value. Popovic, Mullane, Vick and Kobrine[5] also reported a decrease in haemoglobin concentration and oxygen content of the blood that lasted for the entire duration of the anaeshesia produced by phencyclidine. The calcium concentration followed a pattern of change similar to the changes observed in the TPP and albumin values, albumin being the calcium binding factor[9].

The very high values obtained for blood sugar at the initial sampling could be attributed to the effect of adrenaline. The excitement and fear caused by the restraint and injection at the time of administering the anaesthetic agent, probably triggered the liberation of adrenaline into the blood stream. Selye[7] reported that immediately upon exposure to stress a hyperglycaemia occurs, the magnitude of which is largely determined by the hepatic glycogen reserves. An increase in blood sugar, which could be directly correlated with the duration of anaesthesia, has been reported in humans and animals[3]. The sharp decline which occurred in this study was in contrast to this observation. Duration of anaesthesia, depth, manner of induction and the presence or absence of excitement and fear during induction will certainly play a part in determining the blood sugar levels during anaesthesia.

The females had higher blood glucose values than males. They also showed a decrease in potassium with a corresponding increase in sodium at the 30 min sampling. The high blood glucose levels with the subsequent liberation of increased amounts of insulin may have caused some of the circulating potassium to diffuse back into the cells with the sodium moving in the opposite direction[9]. This may also explain the decrease in potassium and the increase in sodium although it is not clear why this occurred only in the females and not in the males.

In a study on anaesthetics administered intramuscularly in *Maca mulatta* it was found that the respiratory rate increased with periods of hyperventilation[4]. However, in another study respiration and blood pressure were not markedly affected except at highly toxic dosage levels when depression of respiration, hypotension and bradycardia were seen[1]. Popovic *et al*[5]. also stated that phencyclidine may potentiate a decrease in the amount of oxygen available to the tissues in pathophysiologic models, since the drug may induce hypoventilation, hypoxaemia and a shift to the right in the oxyhaemoglobin dissociation curve due to respiratory acidosis. The carbon dioxide content of the blood in the baboons included in this study increased significantly suggesting that hypoventilation was more likely than hyperventilation. The elevation of the carbon dioxide content lasted for the entire duration of the experiment with the 60 min value higher than the 30 min value.

The enzymes did not show any marked variations except for the decrease in SGPT over the second 30 min interval in the females. The decrease was, however, so small that no clinical significance could be attached to this alteration.

The initial stress of fear and excitement due to handling and injection would be expected to cause the liberation of relatively large amounts of adrenaline from the adrenal medulla. However, increased corticosteroid secretion does not necessarily follow increased adrenaline levels in the blood and no marked stimulation of the pituitary-adrenal axis could be demonstrated. This would suggest that phencyclidine anaesthesia is not a severe stressor over a period which is normally required for the collection of blood and other specimens.

It is evident from the results and the above discussion that the most outstanding changes demonstrated were haemodilution, increased carbon dioxide content and an increase in blood sugar. The values for certain parameters obtained within three to five min follow-

ing phencyclidine injection showed marked and significant differences as compared to standard values. However, the similarity between standard values and the values recorded at 30 min, is striking. From the results it can be deduced that the administration of the anaesthetic with the concomitant excitement and fear as a result of restraint combined with the effect of the drug itself, can cause considerable alteration in the blood chemistry and haematology. However, the changes are mostly of short duration.

In order to limit variation in the values it would be advisable to follow a strict schedule in collecting specimens by always allowing the same interval between the administration of the anaesthetic agent and the collection of blood. Gentle handling using the same dosage level and the elimination of all unnecessary stress provoking procedures should be part of the routine followed whenever animals are handled for specimen collection.

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## LEAD POISONING IN DOGS

Lead poisoning in humans is becoming more evident in many areas of the wold. This poisoning can also affect dogs. Most usually the affected dog is less than one year of age and the incidence seems to be greater in summer and early fall. The condition is generally found in dogs from older areas of the community where lead based paints and linoleum are readily available. It is also quite interesting that the poodle breed seems to have a high incidence of lead poisoning.

Leukopenia, nucleated erythrocytes and basophilic stippling of the red blood cells is seen in over 90% of the lead poisoning cases, Anemia is variable in up to 30% of the involved cases. Occasionally urinalyses reveals proteinuria and granular casts. However, the blood chemistry is usually within normal ranges.

Diagnosis is facilitated by the evidence of access to lead containing substances such as paints and linoleum. Usually some involvement with the central nervous system can be seen which cannot be attributed to an infectious disease. Treatment involves administration of calcium EDTA at the rate of 50 mg/lb body weight but not to exceed 2 g/day. This dosage should be divided into 4 equal portions, diluted to a concentration of 10 mg/ml of 5% dextrose and administered subcutaneously. Supportive therapy includes: barbiturates, tranquilizers, dexamethasone, calcium salts, fluids, enemas and antibiotics if there is fear of secondary infections.

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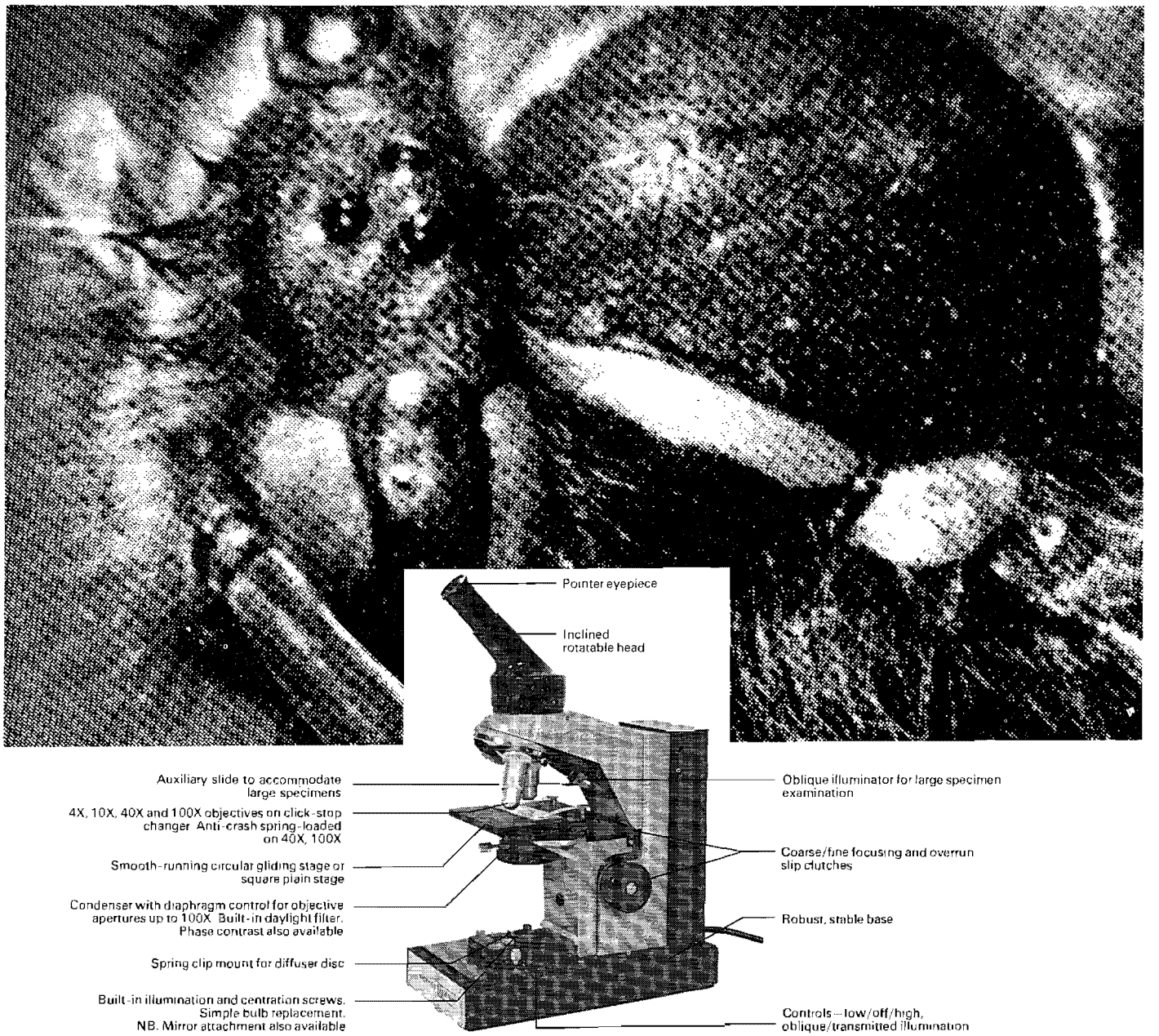


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# PORCINE PARVOVIRUS IN PIG HERDS IN SOUTHERN AFRICA

A. PINI*


## SUMMARY

Evidence of the presence of porcine parvovirus in Southern Africa is given and the diagnostic significance of this finding is discussed. Minor abnormalities in the replication of the virus were observed.


## INTRODUCTION

Breeding disorders are responsible for heavy economic losses in the world pig industry and the assessment of the causes is considered to be among the most difficult problems facing the veterinary profession[15].

Porcine parvovirus and some of the enteroviruses have been isolated in many countries from pig herds affected by infertility, still-birth, abortion and foetal mummification[2 3 5 8 10 12]. Although these viruses may infect the foetus under certain conditions their role as causative agents of reproductive disorders is not yet understood.

Data on the presence in southern Africa of some porcine enteroviruses in breeding herds with a history of reproductive failures were furnished in a previous report[9]. The present communication gives evidence of the presence of porcine parvovirus in the same region.

## MATERIALS AND METHODS

### *Virus*

A lyophilized culture of parvovirus strain 59e/63 was received through the courtesy of Miss S. Cartwright from the Central Veterinary Laboratory, Weybridge, England.

On arrival the virus was reconstituted and primary pig kidney cell cultures were infected when 80% confluent. Haemagglutination (HA) is the criterion for estimating viral replication; cytopathic effects are not used because they are too vague. On the 5th day of incubation, following demonstration of haemagglutinins in the supernatant culture fluid, the entire culture was frozen and thawed three times and harvested. After ultrasonication for 5 minutes a subculture was made. On the 6th day of incubation, when an HA titre of 80 could be demonstrated in the supernate, the culture was frozen and thawed and ultrasonicated as described above. After this treatment the HA test was repeated and the titre attained a value of 320. Virus was stored at -20°C in aliquots of 0,5 ml in sealed ampoules. At this stage an infectivity titration was carried out and a titre of $10^{6,2}$ tissue culture infective doses 50 ($TCID_{50}$) per ml of inoculum was obtained as calculated by the method of Reed and Muench[11].

The identity of the virus stock was checked by the HA inhibition test using a specific anti-serum obtained from the Central Veterinary Laboratory, Weybridge and a rabbit hyperimmune serum produced in our laboratory.

Haemagglutination activity was never detected in uninfected control cultures of pig kidney cells.

### *Cell Cultures*

Primary pig kidney cell cultures were prepared by standard techniques. Eagle's minimum essential medium with 10% serum from adult bovines was used for the production of cell monolayers. In the maintenance medium bovine serum was replaced by 2% foetal bovine serum unless otherwise stated. Monolayers were used when approximately 80% confluent as parvovirus requires actively metabolizing cells.

### *Preparation of Hyperimmune Serum*

Rabbits were inoculated intramuscularly on three occasions at intervals of one week with not less than $3x10^7$ $TCID_{50}$ of virus. They were exsanguinated 3 weeks after the last injection, the serum separated and stored at -20°C. Negative control serum was prepared simultaneously from rabbits inoculated according to the same procedure with uninfected pig kidney cell cultures frozen and thawed 3 times and subjected to ultrasonic treatment for 5 minutes.

The titre of the hyperimmune serum was 640 as estimated by the HA inhibition test.

### *HA and HA inhibition Tests*

The tests were carried out in perspex WHO plates. For the HA test the antigen was diluted in two fold dilution series and used in 0,2 ml volumes. Red blood cells were added to give a final concentration of 0,7% in 0,01 M phosphate buffered saline pH 7,3. Results were read after 60 minutes of incubation at room temperature and the HA titre was expressed as the reciprocal of the highest dilution of antigen in which complete haemagglutination was observed.

For the HA inhibition test sera were inactivated at 56°C for 30 minutes and occasionally also treated with kaolin. Serial two fold dilutions of serum were made to which 4 HA units of antigen were added. The mixtures were incubated for 30 minutes at room temperature and then red blood cells were addded.

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Results were read after incubation for a further 60 minutes.

The inhibiting HA titre of serum is the reciprocal of the serum titre multiplied by the number of HA units used in the test[13].

*Examination of Field Specimens*

Porcine foetal or still-born tissues were tested for the presence of parvovirus only if the foetus or still-born animal was received on ice in good condition, within 3 days after death.

Spleen, liver, kidney, lung, brain and small and large intestine were examined separately. Ten percent organ suspensions were prepared in phosphate buffered saline pH 7,3 and stored at -20°C until required. Large cell debris was removed by centrifugation before infection of the cell cultures. .

Monolayers of primary pig kidney cells were rinsed in phosphate buffered saline and inoculated with 0,2 ml of the appropriate organ suspension. Adsorption was carried out for 60 minutes.

On the 7th day of incubation, in the absence of cytopathic effects, the supernate and cells were harvested. The presence of parvovirus was assessed by the HA test and by the examination of cells stained with haematoxylin and eosin for the demonstration of intranuclear inclusion bodies. Specimens were considered to be negative if haemagglutinins could not be detected after the 3rd consecutive subculture.

## RESULTS

*Replication of the Parvovirus Strain 59e/63*

Maximum HA titres were obtained between the 6th and 8th day after infection of primary pig kidney cell cultures with strain 59e/63. This was 3 to 4 days longer than the time reported by other authors using the same strain of virus[3]. Titres were also lower and did not exceed a level of 320 after freezing and thawing and ultrasonication of the cultures.

To determine whether these results were due to inhibitory factors present in the sera used to maintain the cell system, an experiment was designed in which strain 59e/63 was cultivated in pig kidney cells maintained in the presence of Eagle's medium supplemented with various batches of bovine serum. Nine batches of adult and foetal serum were tested, each incorporated in the medium at concentrations of 1 and 5% respectively. The HA tests were carried out on supernates collected every other day from each group of the infected cultures.

Peak HA titres were never obtained before the 6th day of incubation. In the presence of foetal sera the titres were consistent and of higher levels. With 2 of the 9 adult bovine sera an apparently inhibitory effect on the replication of the virus was observed. The remaining 7 sera gave results comparable to those obtained with the foetal sera. The HA titres obtained with virus cultivated in the presence of 3 different batches of adult or foetal serum are given in Table 1. They are representative of the results obtained.

Infectivity titrations carried out on the same batches of virus obtained on the 10th day of incubation and treated according to the described procedures, confirmed the presence of an inhibitory factor in the systems where adult bovine sera No. 1 and 2 were used.

Table 1: THE INFLUENCE OF BOVINE SERA ON THE REPLICATION OF PARVOVIRUS STRAIN 59e/63

| Bovine serum | | | HA titre | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | & type | Batch No. | Day post infection 2 | 4 | 6 | 8 | 10 |
| 5% | adult | 1 | 0 | 0* | 0 | 10 | 10 |
| | | 2 | 10 | 20** | 20 | 20 | 20 |
| | | 3 | 10 | 20 | 80 | 80 | 80 |
| 1% | adult | 1 | 0 | 0 | 0 | 0 | 10 |
| | | 2 | 10 | 20 | 20 | 20 | 20 |
| | | 3 | 10 | 20 | 80 | 80 | 80 |
| 5% | foetal | 1 | 10 | 20 | 40 | 80 | 80 |
| | | 2 | 10 | 20 | 40 | 80 | 80 |
| | | 3 | 10 | 20 | 80 | 160 | 80 |
| 1% | foetal | 1 | 10 | 20 | 40 | 80 | 80 |
| | | 2 | 20 | 40 | 80 | 80 | 160 |
| | | 3 | 10 | 20 | 80 | 80 | 80 |

* HA test negative with antigen diluted 1/10

** HA titre of supernate

In a further experiment the effect of the incorporation in the maintenance medium of the same foetal and adult bovine sera inactivated at 56°C for 30 minutes was studied. From the data obtained it was concluded that inactivation of the sera did not have any effect on the outcome of the results.

No antibodies to porcine parvovirus could be demonstrated in the 2 bovine sera inhibiting the replication of the virus when they were tested by the HA inhibition test.

*The Influence of Red Blood Cells on the results of the HA Tests*

It has been stated that red blood cells from chickens may vary in their sensitivity towards porcine parvovirus[3,5]. Preliminary investigations at this laboratory revealed that the HA titres obtained with the virus stock using cells from different birds were inconsistent. It was therefore decided to select chickens that would be suitable donors of red blood cells for the test.

Twenty, 14-day old chickens were bled separately and the cells were treated according to the standard procedures. Red cells from 7 of the 20 birds did not show any sign of haemagglutination when tested with the stock antigen at a dilution of 1/10. The cells from 9 birds showed haemagglutination with the antigen at dilutions of 1/20 and/or 1/40. Only the cells of the remaining 4 chickens gave positive haemagglutination with dilutions of antigen of 1/160 and 1/320.

Identical results were obtained when the tests were repeated 3 and 6 months later using the same birds as donors of cells.

Human O red blood cells were also tested. The HA titres were always at least one dilution higher than the titres obtained using chicken cells.

*Serological Survey*

Porcine sera were obtained from herds in South Africa and Rhodesia in which reproductive failures characterized by infertility, still-birth, mummifica-

tion and embryonal death had occurred. Three hundred and ten sera from 68 farms were tested for the presence of antibody to parvovirus by the HA inhibition test. The results are given in Table 2.

Table 2: INCIDENCE OF PARVOVIRUS INFECTION ASSESSED BY THE INHIBITION OF THE HA TEST

| Country | No. of herds | | | No. of sera | | |
|---|---|---|---|---|---|---|
| | tested | positive | negative | tested | positive | negative |
| South Africa | 44 | 40(90%) | 4 | 196 | 165(84%) | 31 |
| Rhodesia | 24 | 12(50%) | 12 | 114 | 45(39%) | 69 |

In 35 of the 40 positive South African herds all the sera tested were positive. The inhibiting HA titres varied between 600 and 40 000 : 72% of them were 10 000 or more, 25% ranged between 2 400 and 9 000 and 3% were below 2 400.

Of the Rhodesian herds 50% were found to be infected and in 7% of these all the sera tested were positive. The range of antibody levels was similar to that found in South Africa : 75% of the sera had titres of 10 000 or more, 23% between 2 400 and 9 000 and in 2% the titres were 1 200 or lower.

*Isolation of Parvovirus from Pathological Specimens*

During the course of this investigation 10 foetuses, originating from 9 herds, were received at this laboratory in a satisfactory condition.

On two occasions a non-cytopathic agent was detected by HA test after the 3rd blind passage in monolayers of pig kidney cells following a total incubation period of 25 days. In a third case a similar agent was obtained on the 2nd passage after an incubation period of 16 days. In all three cases virus was isolated in cell cultures inoculated with organ suspensions prepared from liver, spleen and intestinal tissues. Cells inoculated with brain, lung and kidney suspensions gave negative results.

The three isolates were classified as parvovirus strains on the basis of the inhibition of the HA test by a specific antiserum, the formation of intranuclear inclusion bodies in pig kidney cells and the resistance of the virus to chloroform and ether treatment.

## DISCUSSION

It has been established that porcine parvovirus, like certain porcine enteroviruses, may cross the placenta and infect piglets in utero[4 6 7 10]. The isolation of parvovirus from foetal tissues and boar's semen is relatively recent[3], probably because of certain difficulties in the cultivation and detection of the organism. Although the rate of recovery of parvovirus is low, Cartwright[1] reported that parvovirus is isolated approximately 3,5 times more frequently than the enteroviruses. It has been stated that, because of the rapid autolytic and putrefactive changes occurring in the foetal tissues after death, isolation of these viruses is unsuccessful if attempted 1 to 2 days *post mortem*[15]. In addition, the presence of specific antibodies in the tissues of either the foetus or still-born animal may render the isolation of the causative agent more difficult. To overcome these problems and for practical diagnostic purposes it is suggested that evidence of the presence of the above mentioned viruses be obtained by testing sera from still-born animals or fluids from the thoracic and/or abdominal cavity of foetuses in the late stages of pregnancy for the presence of antibodies[1 15].

In the course of this investigation difficulties were encountered in cultivating the strain 59e/63 of parvovirus. When the results reported here were compared with those published by other authors, working with the same strain of virus, it appeared that our peak HA titres were obtained 3 to 4 days later and were of a lower level[3]. On the other hand the infectivity titres were comparable. The results of the HA titrations were more in accordance with those reported by Johnson[5], working with the same strain of virus in Australia, and other authors using different isolates of parvovirus[8 12].

Johnson[5] reported that some batches of foetal bovine serum used to supplement medium for cell cultures had an inhibitory effect on the development of the HA of parvovirus. Under our experimental conditions no inhibition could be detected when foetal bovine serum was incorporated in the maintenance medium. However inhibition of replication, attributable to adult bovine serum was observed. The inhibitory effect was present when serum was used at concentrations of 1 or 5% and was not influenced by inactivation of 56°C for 30 minutes. This inhibitory effect was apparently not due to the presence of specific antibodies in the serum because bovine parvovirus is not antigenically related to the porcine parvovirus[14]. In addition no inhibition of HA could be detected when the bovine sera were tested.

From these observations and those reported by Johnson[5] it may be concluded that sera should be tested for their ability to support replication of porcine parvovirus, before being used to supplement maintenance media for cell cultures.

Analogous criteria should be applied when red blood cells from chickens are used for the HA test. Many birds may be donors of cells that are not sufficiently sensitive or are otherwise unsuitable for the test.

The results of the serological survey show that the incidence of sera with antibodies to porcine parvovirus is high. In herds with reproductive failures the rate of infection was 80% in South Africa and 40% in Rhodesia. Comparison of these results with those obtained in the survey on enteroviruses reveals that the incidence of infection with parvovirus is at least double that of enteroviruses in South Africa[9].

During this investigation foetal tissues yielded three isolates of parvovirus all antigenically identical to strain 59e/63. The diagnostic significance of these isolations is not clear; virus could have been a cause for the reproductive failures. Experimental observations indicate that parvovirus may have a pathological effect on foetuses when intrauterine infection occurs during early pregnancy[6 10]. It is therefore suggested that ifected semen from boars may be of some epidemiological importance.

The difficulties encountered in assessing the pathogenicity of parvo- and enteroviruses cast doubt on the validity of the diagnostic tests and reaffirm the accuracy of the conclusions drawn by Wrathall[15].

When reproductive failures occur in swine the laboratory tests should only be used in a confirmatory capacity. The diagnosis should be established through an analysis of the herd records, clinical examination and a broad pathological study and not limited to histological examinations of the foetal tissues.

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## Rabbit Embryo Successfully Transplanted

The National Institute of Environmental Health Sciences has reported the successful freezing and birth of rabbit embryos.

After freezing embryos at -196°C from 30 minutes to two weeks, embryos were thawed and grown in culture. More than half the embryos continued to divide and grow for several hours. When implanted, some developed into viable foetuses and normal offspring.

Basic cryobiological information from this system may lead to the successful freezing, storage and transportation of more complex systems, according to the researchers, Harvey Bank of the Medical University of South Carolina, and R.R. Maurer of the above Institue. Procedures similar to theirs may be successful for freezing livestock embryos.

Until now, the only significant success in this direction has been the freezing and implantation of mouse embryos. An experiment with the implantation of a previously frozen cow-embryo proved to be irreproducible.

(*Agricultural Research*, November, 1974; December, 1974, U.S. Department of Agriculture, Washington, D.C. 20250; *Science News*, Feb. 1, 1975, 1719 N Street, N.W., Washington, D.C. 20036)

# THE ELIMINATION OF ALBUMIN, POLYVINYLPYRROLLIDONE AND DEXTRAN FROM THE CIRCULATION IN SHEEP

N.C. OWEN*, A. IMMELMAN** AND DRICKY GRIB **


## SUMMARY

The rates of elimination of iodinated human serum albumin (ALBUMIN-$^{125}$I), polyvinylpyrrolidone (PVP-$^{125}$I); and tritiated dextran (DEXTRAN - METHOXY-$^{3}$H) (mol masses of 69 000 - 72 000, 30 000 - 40 000 and 60 000 - 90 000 respectively) from the circulation of sheep were studied; albumin and PVP initially disappeared from the circulation rapidly having half-life times ($t_{\frac{1}{2}}$) of 10,5 ± 3,7 and 43 ±45 hours respectively. This phase is regarded as being due to equilibration within the initial volume of distribution, the rate being determined primarily by the relative mol. mass of the molecule. Hereafter PVP-$^{125}$I was eliminated considerably faster ($t_{\frac{1}{2}}$ = 176 ±39 h) principally via the kidneys. The limifed data available for dextran-$^{3}$ H suggests that this particular substance is rapidly excreted via the kidneys ($t_{\frac{1}{2}}$ = 9 h)


## INTRODUCTION

The elimination of various large molecular mass substances from the circulation of different animals has been studied and has led to their use as plasma expanders in clinical procedures[1,2]

Thus Giebish, Lauson and Pitts studied the renal excretion of various dextrans by dogs[4] while Hecht and Scholtan[5] followed the excretion of polyvinylpyrrolidone via the kidney of man, dog and rabbit. These substances expand the plasma volume by being retained in the circulation for a period of time[6]. Consequently their elimination from the blood of sheep was compared to that of albumin with a view to their use as markers in capillary permeability studies.

## MATERIALS AND METHODS

### *Experimental animals and labelled substances*

Twelve adult Merino grade whethers were used in these studies. The elimination of albumin was followed in six sheep, that of polyvinylpyrrlolidone in four sheep and dextran in two sheep.

The isotopically labelled substances used and their average molecular masses are:

Iodinated polyvinylpyrrolidone (PVP-$^{125}$I); mol. mass 30 000 - 40 000 (Amersham);

Iodinated human albumin(Alb$^{125}$I); mol. mass 69 000 - 72 000 (Amersham);

Tritiated dextran (Dextran methoxy-$^{3}$H); mol. mass 60 - 000 - 90 000 (New England Nuclear).

### *Experimental procedure*

The sheep were housed individually in metabolism crates designed for the automatic separation of urine and faeces.

At the commencement of each experiment 100μ Ci of isotope were injected into the jugular vein, followed by the collection of venous blood samples 7 ml in volume using heparin as anticoagulant at selected intervals of increasing duration (Fig. 1). Total urine excretion was collected at 12 hourly intervals; the volume noted and a sample taken for analysis. Where iodinated isotope was to be administered, thyroid uptake of labelled iodine was blocked by injecting 10 ml of a 10% w/v Na $I_2$ solution intravenously, 24 hours in advance.

Radioactivity in the case of Alb-$^{125}$I and PVP-$^{125}$I was determined by counting whole blood or urine in a Philips Automatic Welltype Scintillation Detector (Type PW 4003) using a 4,5 x 5,0 cm NaI/Te$^{4}$ crystal. All samples were counted on the same day to obviate correction for decay.

Free-$^{125}$I was estimated in the urine of a sheep injected with PVP-$^{125}$I by passing a quantity of urine through an Amicon Diaflo ultrafiltration membrane with limiting pore size equivalent to 30 000 mol. mass. The ultrafiltrate was then counted as before.

Dextran-$^{3}$H activity was determined by pipetting 0,5 ml. of plasma or 0,1 ml of urine into a 10ml of a commercial scintillation cocktail (Scintisol) and counting in a Packard Tri-carb scintillation spectrometer with Activity Analyser to correct for quenching.

## RESULTS

The relative rates of excretion of the three labelled substances via the kidneys are illustrated by the data given in table I. The blood activity is the mean of the 30 minutes, 8 hour and 24 hour samples taken as corresponding to the period of urine collection. As the figures have little absolute value, a comparative figure is given as the ratio blood counts: urine counts for the three markers.

TABLE 1: RELATIVE EXCRETION RATES OF LABELLED COMPOUNDS OVER A 24 HOUR PERIOD

| Isotope | Activity (c p m) ml | | Ratio |
|---|---|---|---|
| | Blood | Urine | Blood:Urine |
| Albumin$^{125}$I | 27,9 x $10^3$ | 0,97 x $10^3$ | 29,4 |
| PVP$^{125}$I | 28,4 x $10^3$ | 3,8 x $10^3$ | 9,3 |
| Dextran-$^{3}$H | 21,9 x $10^3$ | 583,2 x $10^3$ | 0,38 |


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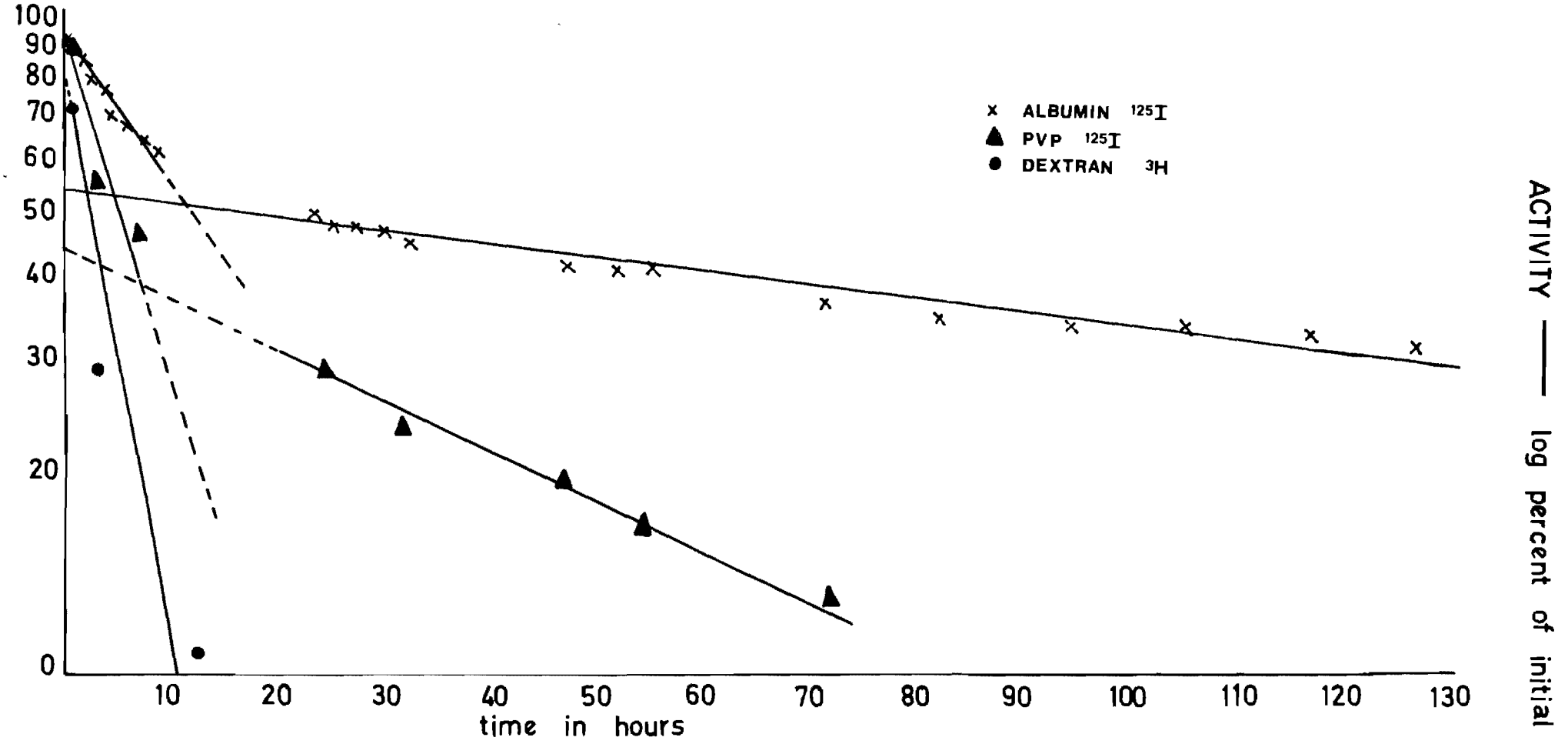


Fig. I DISAPPEARANCE OF ALBUMIN $^{125}I$ PVP $^{125}I$ AND DEXTRAN $^{3}H$ FROM BLOOD OF SHEEF

The ratios show marked differences between the substances regarding the rate of renal excretion over the first 24 hours. Surprisingly, Dextran-$^{3}H$ is excreted very rapidly into the urine when compared to either PVP or albumin. PVP-$^{125}I$ was nevertheless excreted considerably faster than albumin.

The rates of elimination from the blood were estimated by determining the half-life times ($t\frac{1}{2}$) for the different substances. The logarithm of the blood activity was plotted against the time of sampling. Examination of the data suggested a two component disappearance curve, each of which appeared to be almost linear. The first component represented a phase of rapid disappearance which gave the initial blood concentration when extrapolated back to zero time. All subsequent counts were then expressed as a percentage of the initial concentration. Fig. I shows the mean logarithm of the blood activity for all sheep versus the time of sampling.

The results show that Dextran is rapidly eliminated from the blood, PVP less so, while albumin persists at relatively high levels over an extended period of time.

The half-life times ($t\frac{1}{2}$) for the elimination of each of the substances have been estimated from the data obtained from the individual sheep and are shown as means ±1 SD where applicable (Table II)

TABLE II: HALF-LIFE TIMES FOR THE ELIMINATION OF LABELLED COMPOUNDS FROM THE BLOOD OF SHEEP

| Isotopes | Half-life times in hours ** | |
|---|---|---|
| | First component | Second component |
| Albumin-$^{125}I$ | 10,5 ± 3,7 (n=6) | 175,7 ± 3,9 (n=6) |
| PVP$^{125}I$ | 4,3 ± 1,5 (n=4) | 25,5 ± 3,6 (n=4) |
| Dextran-$^{3}H$ | — | 9* |

* Individual values : 8,75 and 9,25 h. n = number experiments
** Values given as means ± 1 standard deviation.

Free iodine was estimated by ultrafiltration in the urine of a sheep injected with PVP-$^{125}I$ and found to be approximately 1,9%. This value was within the specifications of the manufacturer, thus allowing the conclusion that degradation of the PVP had not occurred.

## DISCUSSION

The elimination rates of the three markers (Albumin-$^{125}$ I, PVP-$^{125}I$ and Dextran-$^{3}H$) appeared to be exponential, giving almost linear relationships on a semi-logarithmic plot. Between sheep variation for albumin and PVP elimination was relatively small as evidenced by the biological half-time standard deviations. These two substances disappeared from the circulation in a manner suggesting two distinct processes (a) an initial rapid phase presumably representing equilibration in the final distribution volume; and (b) gradual removal by other means.

Dextran and PVP appear to be eliminated chiefly via the kidneys while albumin is persumably degraded and the free iodine excreted into the urine.

The findings with regard to the slowly excreted component of PVP-$^{125}I$ are essentially in agreement with those of Hecht and Scholtan[5] for dog, man and rabbit. They estimate some 80% of the administered PVP was excreted into the urine during the first 3 days. Our data supports the direct passage of PVP-$^{125}I$ into the urine, giving a biological half-life time of 25 ±3,6 h in sheep.

The nature of the disappearance of labelled albumin from the circulation is very similar to that reported for man[1] and rabbit[3]. However, the half-times required for equilibration in the extracellular fluid of man were three and 24 h using homologous albumin[1] versus an equilibration time of 40 to 60 h for rabbits[3]. Assuming the initial rapid declining phase of activity to represent equilibration in our studies, a biological half-life time of 10,5 ±3,7 h was obtained for heterologous albumin in sheep. The corresponding value for PVP was 4,3 ±1,5 h. The difference in equilibration time may reflect differing final volumes of distribution or the effect of the molecular mass differences between these two substances. The smaller molecule (PVP) passing more rapidly through the capillary walls. Similar differences were observed for albumins and globulins in rabbits[3].

The biological half-life of 176 ±39 h for human serum albumin in sheep corresponds closely to that of 160-230 h for homologous albumin in rabbits[3] and would appear to be a suitable marker for volume distribution and permeability studies. However, antigenicity would preclude repeated use in the same animal. Presumably urinary activity represents free$^{125}I$ detached from the albumins[1,3].

A striking feature of these results is the relatively rapid urinary excretion of dextran with a molecular mass of 60 000-90 000. This contrasts with dextran clearance studies in dogs which showed a rapidly diminishing rate of excretion at mol. masses exceeding 30 000[4]. In view of the use of dextrans as plasma expanders generally[6] this aspect required closer study in the sheep.


## ACKNOWLEDGEMENTS

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# CAUSES OF VARIATION OF COPPER, IRON, MANGANESE, ZINC AND MAGNESIUM LEVELS IN BOVINE LIVERS*

## 3. THE EFFECTS OF LOCALITY

W.J. EHRET,** K.C.W. SANDROCK** AND P.A. BOYAZOGLU***


## SUMMARY

The effects of locality on the copper, iron, manganese, zinc and magnesium levels in 407 bovine caudate lobe liver samples preserved in formalin for differing storage periods were examined. The mineral determinations, expressed on the wet basis (WB), were made by atomic absorption spectrophotometry after wet ashing of the liver. Two hundred and ten of the liver samples were from cattle from one farm (Farm 1) the remaining 197 cattle being from another farm (Farm 2).

The copper, iron and magnesium levels were taken as indicative of the hepatic concentrations at slaughter. Locality had a significant effect ($P < 0,05$) on the copper, iron and magnesium levels. All copper levels on Farm 1 fell well below the accepted minimum (33,0 mg/kg). The deficiency appeared to be secondary with the possible implication of sewage effluent.

In terms of biological variation the different iron levels appeared of minor importance and no inverse relationship was found between iron and copper.

The manganese and zinc levels were interpreted with caution due to the significant differences reported in their hepatic concentrations after six months of storage in formalin. Extremely high zinc levels in individual animals could have been associated with sewage effluent.


## INTRODUCTION

The liver is the main storage organ for copper and its copper concentration has been found to vary enormously[35]. Low levels of copper in the livers of cattle are the result of a primary deficiency, when the diet is inadequate, or a secondary (conditioned) deficiency when the dietary intake is sufficient, but the utilization of the copper is impeded, for example by the interaction of molybdenum and sulphate[5 27 31 32]. Copper deficiency in cattle has been associated with unthriftiness, anaemia[26 31], a low incidence of spontaneous bone fractures[31] and neonatal ataxia[31]. Depigmentation and defective keratinization of hair, fibrosis of the myocardium with associated sudden death, severe diarrhoea and low fertility[2 26 31] have also been described in copper deficient areas. More recently copper supplementation in cattle has produced significant increases in conception rates[1], reduced intercalving periods[15] and improved semen quality[14].

The liver is also the main storage organ for iron[35] and its content is affected by an interaction between the developmental or productive status and the diet of the animal concerned, but may also be due to the influence of certain pathological conditions. Iron-deficiency anaemia leads to a diminution in the amount of iron stored in the liver. This primary deficiency has been recorded in calves consuming an exclusive milk diet[17] and there is a positive correlation between the blood haemoglobin level and liver iron in calves[28]. There is, however, no convincing evidence that iron deficiency ever occurs in grazing stock under natural conditions, except possibly in circumstances involving severe blood loss or a disturbance in iron metabolism, as a consequence of parasitic infestation or disease[5 32].

Manganese is not concentrated in any particular organ or tissue. The concentrations in the liver are, however, higher than most other tissues and can be raised or lowered with varying manganese intake. The manganese storage capacity of the liver is limited when compared with the remarkable capacity of this organ to accumalate iron and copper[31 35]. Although a primary manganese deficiency is rare under farm conditions[5], a deficiency of manganese in cattle may cause poor growth, skeletal abnormalities and depressed or disturbed reproductive function[3 9 25 31 32]. Improved fertility has been recorded with manganese supplementation in cattle grazing on manganese deficient herbage[36].

According to Underwood[32] the capacity of the animal to store zinc in any of its organs other than bones, is extremely limited so that animals do not normally carry large reserves of zinc. More recently van Leeuwen and van der Grift[33] and Miller, Blackmon, Gentry and Pate[19] have shown that high dietary levels of zinc give rise to large increases in bovine liver zinc levels. As a state of zinc deficiency develops there is usually, but not invariably, a small decline in the concentration of zinc in the liver and certain other tissues[32]. In cattle, zinc deficiency has been associated with subnormal growth, alopecia and parakeratosis particularly of the muzzle, ears, neck, genitalia, back of the hind legs and knee-folds, a stiff gait and swelling of the hocks and knees and retarded testicular development[4 20 21 24 32 33]. Zinc supplementation has been associated with a marked improvement in conception rate in cows[22].

A lowered serum-magnesium concentration is implicated in the metabolic hypomagnesaemic tetanies of cows and calves. Some workers claim that the magnesium content of milk, soft tissue and bones of cows with hypomagnesaemic tetany remain within normal limits[32 34]. Others claim that although there is no large mobilizable store of magnesium in the body, the reserves which do exist in the bones and soft tissues can be of importance under stress situations[5]. Low

* Based upon a dissertation submitted by W.J. Ehret to the University of Pretoria in partial fulfilment of the requirements for the degree M. Med. Vet. (Zootech.)

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magnesium intake has been associated with the calcification of certain soft tissue, in particular blood vessels, as well as renal lesions, in laboratory animals[18], calves[8] and more recently in sheep[6]. From his investigations in sheep, Boyazoglu[6] found that the occurrence of these lesions was not only directly correlated with low magnesium levels in the diet but also with low magnesium levels in the liver. Furthermore in his studies on calcium and phosphorus inadequacy, Boyazoglu[6] has encountered high magnesium levels in the liver in the presence of normal dietary magnesium intakes.

An earlier pilot investigation conducted on 80 beef cattle owned by the Johannesburg City Council, in which the analysis of liver samples for copper, zinc and manganese concentration was undertaken brought to light an apparent copper deficiency. This deficiency was more marked in cows from one of the two farms, from which the samples were drawn[10]. In view of the implications regarding decreased productivity, more extensive investigations were instituted so that corrective procedures could be applied should the results of the pilot trial be confirmed. It was decided to include iron and magnesium assays in the studies.

## MATERIALS AND METHODS

A total of 407 liver samples from cross-bred beef cattle of various ages were taken at slaughter and submitted to a laboratory for analysis for copper, iron, manganese, zinc and magnesium content[29]. The cattle, slaughtered over a period of 15 months, came from two intensive beef enterprises which were ancillary to sewage and waste-water purification plants operated by the Johannesburg City Council.

### *EXPERIMENTAL ANIMALS*

Of the 407 liver samples 210 were from animals from Farm 1 situated 40 kilometres north of Johannesburg. Apart from the areas occupied by the purification plant and that used in the production of preserved communal cattle feed, the farm is divided into five, separate cattle sections. Three of these, namely 1, 2 and 3 are cow breeding and calf raising sections. Section 4 comprises a heifer breeding and young stock section and is the section to which the weaned calves from sections 1, 2 and 3 are sent at approximately 7 months of age. Section 5 is a separate feedlot.

The remaining 197 liver specimens were from animals from Farm 2 situated 24 kilometres south of Johannesburg. As with Farm 1 a considerable portion of Farm 2 is not grazed by cattle. The five cattle sections are once again strictly separated and also comprise three cow breeding and calf raising sections (sections 1, 2 and 3), a heifer breeding and young stock section (section 4) and a separate feedlot (section 5).

The origin of the 407 liver samples is given in table 1.

All animals with the exception of the weaners, steers and 10 breeding heifers from section 1 of Farm 2 which were recently transferred from section 4, had been in their sectional localities for at least 6 months prior to slaughter.

The breeding females slaughtered were animals culled for failure to conceive, but because a very strict

Table 1: ORIGIN OF BOVINE LIVER SAMPLES

| Type | FARM 1 Section | | | | | FARM 2 Section | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Cows | 50 | 59 | 10 | | | 59 | 42 | 48 | | |
| Breeding heifers | | | | 12 | | 10 | | | 8 | |
| Maiden heifers | | | | 20 | | | | | 20 | |
| Weaners | | | | 32 | | | | | | |
| Steers | | | | | 27 | | | | | 10 |
| Total | 210 | | | | | 197 | | | | |

culling procedure was in force it should not be concluded that all the cows were infertile. The maiden heifers and weaners were culled because they fell below the operative group selection indices calculated mainly on a daily weight gain. The steers were slaughtered when ready for market.

During the sampling period the cattle population of Farm 1 averaged 3 479 with an average of 1 825 breeding females. During the same period the average population on Farm 2 was 4 187 with 2 002 breeding females. The respective production figures for Farms 1 and 2 for the three breeding seasons from which the sampled cows were drawn is given in Table 2. The number of calves weaned, over the total number of females bred, is expressed as a percentage. Breeding on both farms was by artificial insemination.

Table 2: PRODUCTION OF CALVES ON FARMS 1 AND 2, FOR THE BREEDING SEASONS OF SAMPLED BREEDING CATTLE.

| Breeding Season | Calf Production % | |
|---|---|---|
| | Farm 1 | Farm 2 |
| June/July/August 1968 | 58,8 | 71,0 |
| Nov./Dec. 1968 Jan. 1969 | 57,7 | 79,0 |
| June/July/August 1969 | 61,6 | 73,0 |

### *MANAGEMENT AND FEEDS*

Extensive use is made of effluent flood irrigation in the production of the cattle feed grown on these farms. The effluent used on Farm 1 is mainly from domestic sewage with the direction of flow from section 4 through sections 2 and 1 and finally to section 3. The irrigation effluent used on Farm 2 comes from two purification works, one of which handles both industrial and domestic waste-water and supplies the irrigation effluent for sections 4 and 1. The other works handles mainly domestic sewage and supplies first section 2 and then section 3.

The feeds produced on both farms were similar. The pastures consisted predominantly of rye-grasses (a mixture of perennial rye-grass *(Lolium perenne)* Italian rye-grass *(L. multiflorum)* and tetraploid rye *(L. multiflorum sel.)*, a mixture of rye-grass, small areas of clover *(Trifolium spp.)* and particularly on Farm 2, fescue grasses *(Festuca spp.)* and cocksfoot *(Dactylis glomerata L.)*. In addition, large *Eragrostis curvula* pastures, and particularly on Farm 2, kikuyu pastures *(Pennisetum clandestinum)*, plus small areas of naturally occurring veld grasses were available. *E. curvula* hay and a limited amount of

lucerne hay *(Medicago sativa)* was produced. Maize *(Zea mays)* and some hybrid sorghum species were grown and ensiled.

The only additional feed used during the period of this study was a maize/sorghum brewers grain mixture.

A lick consisting of equal quantities of salt (NaCl) and dicalcium phosphate to which 0,25 per cent copper sulphate and 0,125 per cent cobalt sulphate was added was continually available to all animals. The intake of the lick was not monitored and an adequate intake is questionable owing to its low acceptability.

Drinking water from a domestic source was provided in all overnight camps and feeding pens, but grazing animals on occasion had access to effluent water from the irrigation canal and holding dam system on the two farms.

The animals in both farms feedlots were on zero grazing. All other animals had access to pastures for the bulk of their nutritional requirements. The nutritional requirements were however controlled and were not on an *ad libitum* basis.

The access to grazing is important in so far as both farms were infested with liver fluke (mainly *Fasciola hepatica*) and consequently grazing animals were exposed to varying degrees of infestation depending upon the locality.

## *COLLECTION OF LIVER SAMPLES*

All liver samples were removed at the time of evisceration which was approximately 45 minutes after stunning and bleeding. Annotation of degree of liver damage due to fascioliasis was made and only those livers not showing extensive damage were sampled. Each sample in the form of an approximate 20 g blunt ended wedge was taken from a portion of caudate lobe which appeared normal. The sample was immediately placed in a glass bottle containing sufficient 10 per cent formalin to cover the specimen completely. The formalin solution was prepared from laboratory reagent formaldehyde solution (37 to 41 per cent (W/V) diluted with distilled water. After collection the bottles were tightly sealed and stored until required for the analysis of the various elements[29]. The period of storage varied from 4 to 19 months. The methods for the determination[29] and the dispersion of the metals in the liver together with the effects of storage in formalin on the mineral concentrations and moisture content of liver samples have been previously described[30].

The mineral concentrations are expressed on the wet basis (WB)

## *ANALYSIS OF DATA*

Data pertaining to the mineral content of the livers was arranged as detailed below. The possible interactions of the age of animal, time of slaughter and varying degrees of liver damage on the mineral concentrations in the livers were ignored.

Comparisons were made between:-

(i) the overall results of the two farms;
(ii) the composite results of the cows of the two farms;
(iii) the composite results of the breeding females of the two farms
(iv) the individual cow and heifer sections within each farm.

The 10 recently introduced breeding heifers from section 1 on Farm 2 were excluded from the comparisons made in sections (iii) and (iv) above.

Analysis of variance (Anova) was used to determine the statistical significance of the above comparisons. In the formal Anova the analysis terminates with the calculation of the F-ratio and it is left to the experimenter to determine which treatments led to the rejection of the null hypothesis (Ho) if this was the case. The computer however was programmed to perform the Scheffe multiple comparison procedure (S-method) and in this way isolate the out of range means.

The comparisons were tested at the 95 per cent level of confidence ($P < 0,05$).

## RESULTS

The comparison between the overall results of the 210 cattle from Farm 1 and the 197 cattle from Farm 2 (Table 3) showed that the levels of copper, iron and zinc of Farm 1 were significantly lower ($P < 0,05$) than those of Farm 2.

Table 3: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg BETWEEN FARMS 1 AND 2

| Farm | N | Mineral concentrations mg. per kg. Wet Basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cu | | Fe | | Mn | | Zn | | Mg | |
| | | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| 1 | 210 | 15,2 | 13,6 | 120,1 | 63,7 | 3,6 | 1,0 | 39,9 | 26,6 | 136,4 | 26,1 |
| 2 | 197 | 32,1 | 20,0 | 133,2 | 46,6 | 3,7 | 1,1 | 51,3 | 58,5 | 135,0 | 35,3 |
| Comparison P<0,05 | | * | | * | | N.S. | | * | | N.S. | |

Cu = copper. Fe = iron. Mn = manganese. Zn = Zinc. Mg = Magnesium
N = Number of samples $\bar{X}$ = Mean; S.D. = Standard deviation;
* = Significant (at the 95 per cent level of confidence);
NS = Not significant (at the 95 per cent level of confidence)

The comparison between the composite results of the cows of the two farms (Table 4) showed that the 119 cows of Farm 1 had significantly lower ($P < 0,05$) copper and iron levels than the 149 cows from Farm 2.

Table 4: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF THE COWS OF FARMS 1 AND 2

| Farm | N | Mineral concentrations mg/kg. Wet Basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cu | | Fe | | Mn | | Zn | | Mg | |
| | | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| 1 | 119 | 11,1 | 11,6 | 107,2 | 34,4 | 3,6 | 1,0 | 41,3 | 32,1 | 128,7 | 23,0 |
| 2 | 149 | 30,7 | 20,4 | 133,4 | 48,0 | 3,6 | 1,2 | 49,8 | 61,1 | 130,5 | 23,4 |
| Comparison $P<0,05$ | | * | | * | | N.S. | | N.S. | | N.S. | |

The comparison of the composite result of the breeding females of Farm 1 and Farm 2 is given in Table 5. It was found that the inclusion of the breeding heifers of section 4 did not alter, to any degree, the figures shown in Table 4 above. Once again the copper and iron results of Farm 1 were significantly lower ($P < 0,05$) than the results of Farm 2.

Table 5: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF THE COWS AND BREEDING HEIFERS OF FARMS 1 AND 2

| Farm | N | Mineral concentrations mg/kg. Wet Basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cu | | Fe | | Mn | | Zn | | Mg | |
| | | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| 1 | 131 | 10,7 | 11,3 | 107,2 | 33,1 | 3,5 | 1,0 | 41,4 | 32,0 | 128,8 | 22,6 |
| 2 | 157** | 31,2 | 20,4 | 132,9 | 47,2 | 3,6 | 1,2 | 52,4 | 61,8 | 131,1 | 23,5 |
| Comparison $P<0,05$ | | * | | * | | N.S. | | N.S. | | N.S. | |

** 10 breeding heifers ex section 1 of Farm 2 not included.

The results and comparisons of the cows from sections 1, 2 and 3 and the heifers from section 4 of Farm 2 are given in Table 7. Significant diferrences ($P < 0,05$) occurred in the copper, manganese and magnesium levels. The 59 cows from section 1 had the lowest copper levels which were significantly lower than the 42 cows from section 2, the 48 cows from section 3 and the 28 heifers from section 4. However, there were no significant differences between the copper levels of section 2, 3 and 4. The 28 heifers had the highest manganese levels which were significantly ($P < 0;05$) different from the results of sections 1 and 3 but not different from section 2. The magnesium levels for the 28 heifers were significantly higher than those of the three cow sections. The levels for zinc, although ranging from a mean of $37,3 \pm 22,6$ mg/kg for section 3 to a mean of $73,2 \pm 60,1$ mg/kg for the 28 heifers were not significantly different ($P < 0,05$). The levels for iron showed little variation and were not statistically different ($P < 0,05$).

The results and comparisons of the cows from sections 1, 2 and 3 and the breeding and maiden heifers from section 4 of Farm 1 are given in Table 6. The only significant difference ($P < 0,05$) between the sections occurred in the copper and magnesium levels. The 59 cows from section 2 had the lowest copper levels, which were significantly different ($P < 0,05$) from the levels of the 50 cows from section 1 and the levels of the 10 cows from section 3, but were not significantly different from the levels of the 32 heifers. The 32 heifers had significantly higher ($P < 0,05$) magnesium

Table 6: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF COWS FROM SECTIONS 1, 2 AND 3 AND HEIFERS FROM SECTION 4 OF FARM 1

| Farm 1 Sections | N | Mineral concentrations mg/kg. Wet Basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cu | | Fe | | Mn | | Zn | | Mg | |
| | | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| 1 | 50 | 13,5 | 11,7 | 100,4 | 26,6 | 3,5 | 1,1 | 33,1 | 7,9 | 131,3 | 24,5 |
| 2 | 59 | 7,4 | 8,6 | 114,3 | 40,1 | 3,7 | 0,9 | 46,5 | 43,8 | 126,1 | 22,8 |
| 3 | 10 | 21,3 | 17,9 | 99,5 | 25,3 | 3,7 | 0,5 | 51,6 | 13,1 | 131,7 | 16,0 |
| 4 | 32 | 11,2 | 8,4 | 102,0 | 15,2 | 3,7 | 1,0 | 42,2 | 24,4 | 152,4 | 28,2 |
| Comparison $P<0,05$ | | 1 * 2<br>3 * 2 | | N.S. | | N.S. | | N.S. | | 1 * 4<br>2 * 4 | |

Table 7: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF COWS FROM SECTIONS 1, 2 AND 3 AND HEIFERS FROM SECTION 4 OF FARM 2

| Farm 2 Sections | N | Mineral concentrations mg/kg. Wet Basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cu | | Fe | | Mn | | Zn | | Mg | |
| | | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| 1 | 59 | 19,5 | 13,7 | 131,7 | 55,8 | 3,6 | 1,3 | 65,8 | 88,3 | 132,0 | 26,7 |
| 2 | 42 | 35,6 | 18,3 | 136,5 | 49,8 | 3,9 | 1,0 | 41,5 | 35,0 | 133,0 | 23,0 |
| 3 | 48 | 40,2 | 22,7 | 132,6 | 35,1 | 3,2 | 1,1 | 37,3 | 22,6 | 126,3 | 19,0 |
| 4 | 28 | 33,4 | 16,8 | 135,6 | 51,8 | 4,6 | 0,9 | 73,2 | 60,1 | 157,1 | 25,2 |
| Comparison P<0,05 | | 1 * 2<br>1 * 3<br>1 * 4 | | N.S. | | 1 * 4<br>3 * 4 | | N.S. | | 1 * 4<br>2 * 4<br>3 * 4 | |

## DISCUSSION

The copper, iron and magnesium levels were taken as indicative of the hepatic concentrations at slaughter[30]. However, the manganese and zinc levels were interpreted with caution due to the significant differences in their hepatic concentration reported after 6 months of storage in formalin[30].

### *COPPER LEVELS*

Blood and Henderson[5] state that normal concentrations of copper in the livers of cattle should be above 100 mg/kg when calculated on the dry basis (DM) but are usually above 200 mg/kg (DM). These values have been confirmed by Underwood[32] who states that concentrations of copper in the livers of normal adult cattle are consistently high, within a range of 100 to 400 mg/kg on the dry basis with a high proportion lying between 200 and 300 mg/kg. When these values are converted to a wet basis (using a 66 per cent moisture content[30]) it would appear that normal levels should all be above 33 mg/kg (100 mg/kg DM) with the majority being between 66 and 99 mg/kg (200 to 300 mg/kg DM) Boyazoglu, Barrett, Young and Ebedes[7] in their recently completed survey of 190 bovine livers collected from South Africa found that the mean copper content was 48,8 ±31,9 mg/kg on the wet basis.

In terms of the above results it is quite obvious that all the copper levels of Farm 1 fell well below the accepted minimum. The overall mean of 15,2 ±13,6 mg/kg was less than half of this value. The mean of the breeding females was less than a third and the mean of the 59 cows from section 2 (7,4 ±8,6 mq/kg) was less than a quarter of the accepted minimum level. Whereas the values for the overall farm, cows and breeding females of Farm 2 can be regarded as just falling into the minimum accepted level, the sectional comparisons showed that the levels of the 59 cows from section 1 (mean 19,5 ±13,7 mg/kg) were well below normal limits.

As stated in the introduction, various workers have associated a copper deficiency with certain clinical manifestations in cattle. Clinical signs observed amongst cattle on Farm 1 suggest that they may have been associated with a copper deficiency. Unthriftiness, harsh stary hair coat, scours and impaired fertility were seen. As a parameter of impaired fertility one can compare the low overall calf production percentage of Farm 1 relative to Farm 2. Although some black coated animals had a slight reddish tinge, which could have been genetic, no specific symptoms were observed of copper deficiency. In fact the clinical signs observed were indistinguishable from those resulting from unbalanced nutrition or from helminthiasis, both of which were distinct possibilities. However, the low levels of copper demonstrated in the liver confirmed that copper was in fact involved.

The cumulative data of this investigation and the investigation into the effect of the age of the animal on the mineral concentrations[11] [12] suggest that the low copper levels were secondary (induced) rather than primary. Blood and Henderson[5] point out that extensive deposits of haemosiderin can be found in the liver, spleen and kidney in most cases of primary copper deficiency. However, no inverse relationship between iron and copper was observed in low copper status animals. In addition the demonstration that the younger animals on Farm 1 had significantly higher ($P < 0,05$) copper levels[11] [12] adds further weight to the deficiency having been an induced one. According to Blood and Henderson[5] "Apart from falling disease which occurs only in adult cattle, young animals are much more susceptible to primary copper deficiency than are adults. Calves on dams fed deficient diets may show signs at 2 to 3 months of age. As a rule the signs are severe in calves and yearlings, less severe in 2 year-olds and of minor degree in adults."

Of interest as possible aetiological factors of an induced copper deficiency was the presence of fair amounts of sulphates and nitrates in the sewage effluent[23]. The interaction of sulphate with molybdenum in the inducement of a copper deficiency has been mentioned. Nitrogen fertilization has been shown to bring about a decrease in copper concentration in plants[27] and the presence of nitrates in the irrigation effluent plus the liberal use of inorganic nitrogen fertilizer on the farm could have aided the manifestation of copper deficiency amongst the cattle.

The importance of balanced nutrition and elemental interaction as given in Mulder's interaction chart[27] should be borne in mind. More detailed analysis of the constituents of the effluent and various forages, and of the animals would have been necessary to confirm whether the deficiency was in fact induced, and if so, by what possible factors.

The association between the copper levels of the sections on Farm 1 with the flow of the irrigation effluent is interesting. With the exception of section 4 which was closest to the works, the copper levels were

found to increase with increasing distance from the works. This relationship would seem to support the possible presence in the effluent of some conditioning factor, which decreased with increasing distance from the purification works. In this regard it can be mentioned that although the sulphate concentration of the effluent remained fairly constant, the nitrate content decreased with increasing distance from the purification works[23]. The levels of copper in the livers of cows from sections 2, 1 and 3 (given in order of proximity to the purification works) were 7,4 ±8,6 : 13,5 ±11,7 and 21,3 ± 17,9 mg/kg respectively. However, section 4, which was closest to the sewage works had higher copper concentrations (11,2 ±8,4 mg/kg for 32 sampled heifers) than the section next in line (section 2: 7,4 ±8,6 mg/kg for 59 cows). If the other animals of section 4, namely the weaners[11] had been taken into consideration, the values would have been even higher. A possible explanation for these relatively higher levels, appears to be a combination of age of animal and their brief stay in the locality. Of further interest, in support of the possible incrimination of the sewage effluent with the low concentrations of copper in the livers, is the fact that the steers (section 5) on both farms, but particularly on Farm 1, had consistently higher liver copper levels than the other sub-groups[11]. As stated earlier these animals were on zero grazing and did not come into direct contact with the sewage effluent.

## *IRON LEVELS*

Iron deficiency states are rare in farm animals. The iron content of herbage plants although very variable as a consequence of species differences and soil effects is invariably much higher than the normal requirement for this element by the grazing animal. In addition the intake of soil under most grazing conditions, provides considerable opportunity for the grazing animal to obtain additional iron[32].

Boyazoglu *et al.* found the iron content of bovine livers to be 149 ±82,2 mg/kg (WB). These values together with the means for Farms 1 and 2 of 120,1 ±63,7 mg/kg and 133,2 ±46,6 mg/kg, as well as the sectional variations of 99,5 ±25,3 mg/kg for the 10 cows from section 3 of Farm 1 up to the mean of 136,5 ±49,8 mg/kg for the 42 cows of section 2, Farm 2, would all appear in the light of the earlier remarks to fall within normal limits. In terms of this apparent wide biological variation the demonstration of statistically significant ($P<0,05$) inter-farm differences therefore appears meaningless. Although variation in the concentrations in individual animals was extreme (39,9 to 729,9 mg/kg), the majority fell within, or close to, the above mean values[11].

Of interest is the examination of the copper : iron relationships both on group level and on individual animal level[11]. Marston, Lee and McDonald[16] demonstrated extensive deposits of iron in the liver and other tissues of sheep confined to copper deficient grazing. More recently Boyazoglu *et al.* drew attention to a similar inverse relationship between copper and iron liver levels in certain individual cattle. However, the examination of the various mean values given above show that the lower copper values are invariably coupled with the lower iron values. Only in the case of the sectional comparisons on Farm 1 were the lowest copper levels (mean 7,4 ±8,6 mg/kg) coupled to the highest iron levels (mean 114,3 ±40,1 mg/kg). Furthermore perusal of the data of individual animals showed no consistent relationship[11].

## *MANGANESE LEVELS*

Primary manganese deficiency is uncommon under farm conditions. However, a manganese dificient diet usually results in a lowering of the limited amount stored in the body, located mainly in the bones and to a smaller extent in the liver and other tissues[32]. According to Underwood[32] the liver levels of manganese are useful but not entirely reliable indicators of manganese deficiency, unless the deficiency is severe. Gessert, Berman, Kastelic, Bentley and Phillips[13] state that the livers of cattle generally contain 8 to 10 mg/kg manganese on the dry basis (2,7 to 3,3 mg/kg WB) whereas Blood and Henderson[3] state that the manganese content of the liver in normal animals is about 12 mg/kg on the dry basis (4 mg/kg WB) and about 8 mg/kg (DM) (2,7 mg/kg WB) in calves. However Boyazoglu *et al.* found a mean of 8,1 ±2,6 mg/kg (WB) for 188 bovine livers, which included the bulk of the 80 livers from Farms 1 and 2 (mean 9,3 ±2,9 mg/kg) examined in the pilot investigation[10].

The various mean values for Farms 1 and 2 in this study fell within the range of normality given by Gessert *et al.*[13] and Blood and Henderson[3]. However, the range for individual animals was quite wide, from 1,2 mg/kg to 10,2 mg/kg[11].

The investigations into the effect of prolonged storage in formalin on manganese concentrations revealed a statistically significant increase ($P\pm 0,05$)[30]. This is 'however' difficult to equate with the variations of individual animals[11]. Groups from different farms sampled at the same time and therefore subjected to similar storage conditions showed extreme variation. This was well illustrated in a group from Farm 2 where the 19 animals had a mean of 3,65 mg/kg and varied from 2,0 to 9,2 mg/kg[11]. Although this variation was extreme, the pattern of high and low within a group was constant[11]. This was interpreted as indicating an inconsistent loss or gain of manganese due to storage in formalin and it was concluded that the various comparisons were valid.

The statistically significant ($P<0,05$) sectional differences on Farm 2 where the heifer section had the highest levels (mean 4,6 ±0,9 mg/kg) and cow section 3 the lowest (mean 3,2 ±1,1 mg/kg) were interpreted as insignificant in terms of accepted normal variation

## *ZINC LEVELS*

As stated earlier there is definite evidence of a naturally occurring zinc deficiency in cattle.

Gessert *et al.*[13] reported a mean of 125 mg/kg zinc on the dry basis for the livers of 30 milking cows (42 mg/kg on the wet basis) and van Leeuwen and van der Grift[33] after working on zinc metabolism concluded that normal liver values for cattle lay between 100 to 200 mg/kg (DM), that is between 33 and 66 mg/kg (WB). However Boyazoglu *et al.*[7] recorded a mean of 158,0 ±75,9 mg/kg (WB) for 189 cattle in South Africa. Included in the latter analyses are the bulk of the 80 livers from Farms 1 and 2 (mean 103,3 ±43,0 mg/kg) examined in the pilot investigation[10] Thus, as with

manganese, the current zinc levels on the farms were lower than those demonstrated in the pilot investigation[10].

However, it is difficult to interpret the values demonstrated in this study in view of the significant ($P < 0,05$) loss of zinc content shown to occur during 6 weeks to 6 months storage in 10 per cent formalin[30]. Thus no conclusions were drawn, even though no group levels were found to be below the 33 mg/kg (WB) given by van Leeuwen and van der Grift[33] as the normal minimal value. No clinical signs indicative of a zinc deficiency were observed on either farm.

In contrast to the variation of manganese concentrations, the groups on the farms showed consistent zinc levels with the particular exception of some groups from sections 1 and 4 of Farm 2[11]. This is interesting in view of the fact that these sections were the only sections supplied by the sewerage works handling the greatest volume of industrial effluent. Some of the levels demonstrated on these two sections were extremely high (up to 501,8 mg/kg). This observation plus the marked variation encountered may have been associated with the zinc content of the effluent.

## *MAGNESIUM LEVELS*

Boyazoglu *et al.*[7] in their analysis of 188 bovine livers for magnesium content demonstrated a mean of 208 ±60,7 mg/kg on the wet basis. The levels demonstrated on Farms 1 and 2 were, however, lower, (overall farm means: Farm 1 136,4 ±26,1 mg/kg and Farm 2 135,0 ±35,3 mg/kg with the overall mean of the cows and breeding females showing hardly any deviation from the overall mean). However, the sectional comparisons within a farm showed that the heifers on both farms had the significantly highest levels ($P < 0,05$). Although the variation in the levels of magnesium of individual animals was extreme, from 46,2 to 463,0 mg/kg (a breeding heifer from section 1 of Farm 2), the majority fell close to, or within their respective mean values[11].

Boyazoglu[6] has found high concentrations of magnesium in the liver to be indicative of inadequate calcium : phosphorus nutrition and in terms of this finding the demonstrated sectional differences may indicate that the calcium and phosphorus intake of the heifers was relatively inadequate despite the fact that all magnesium levels fell below those of Boyazoglu *et al.*[7]


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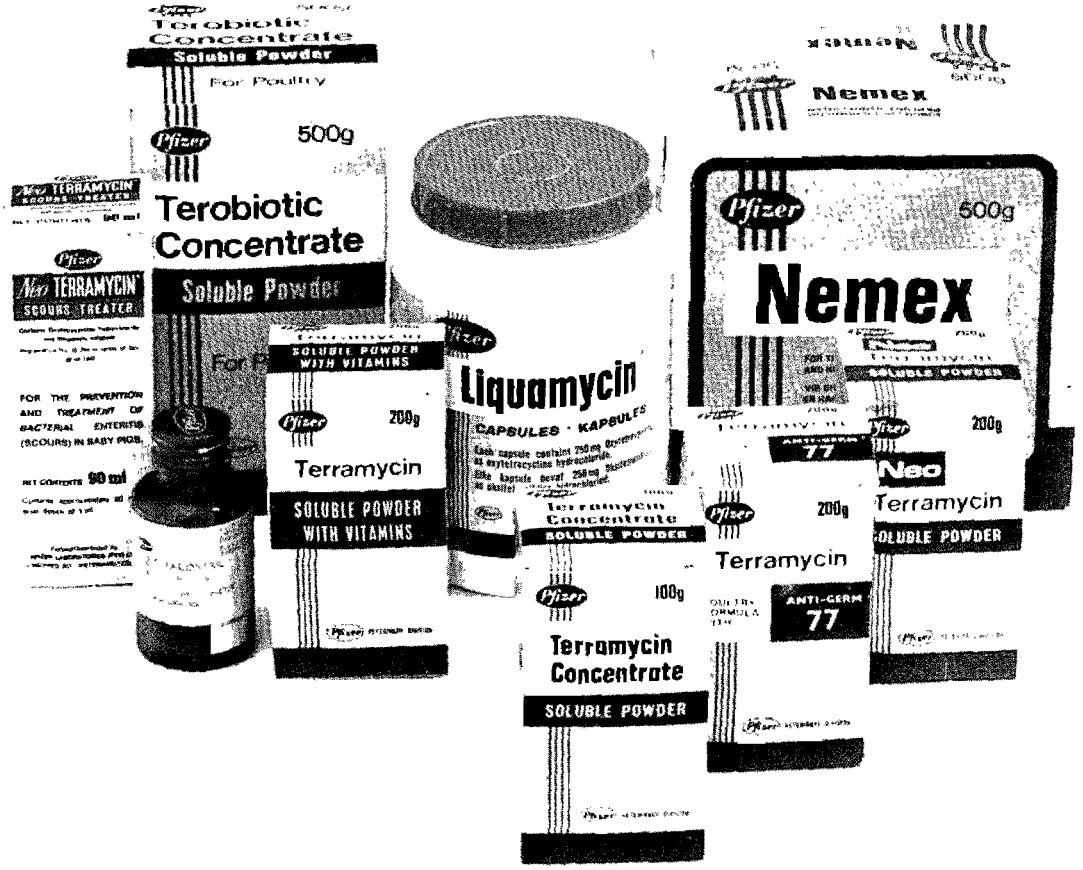


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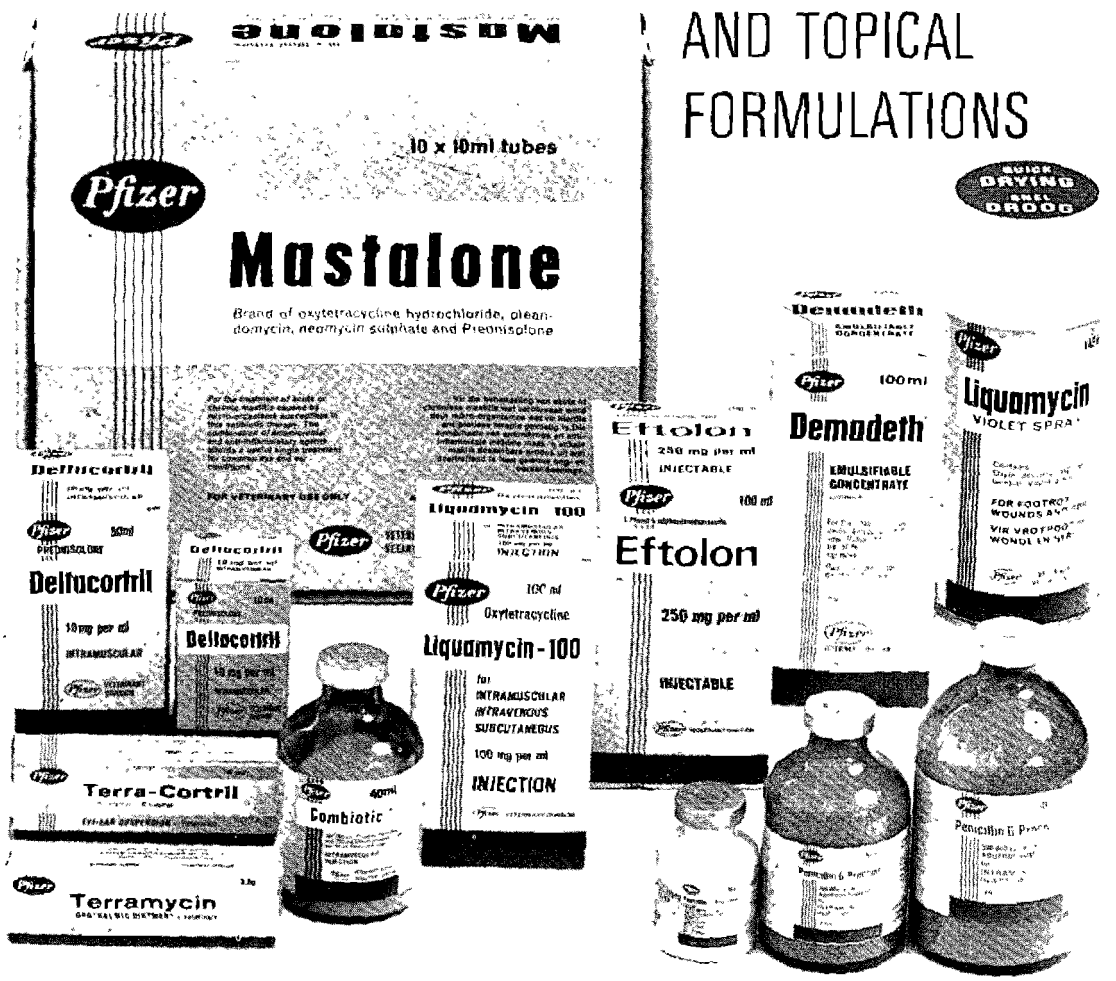


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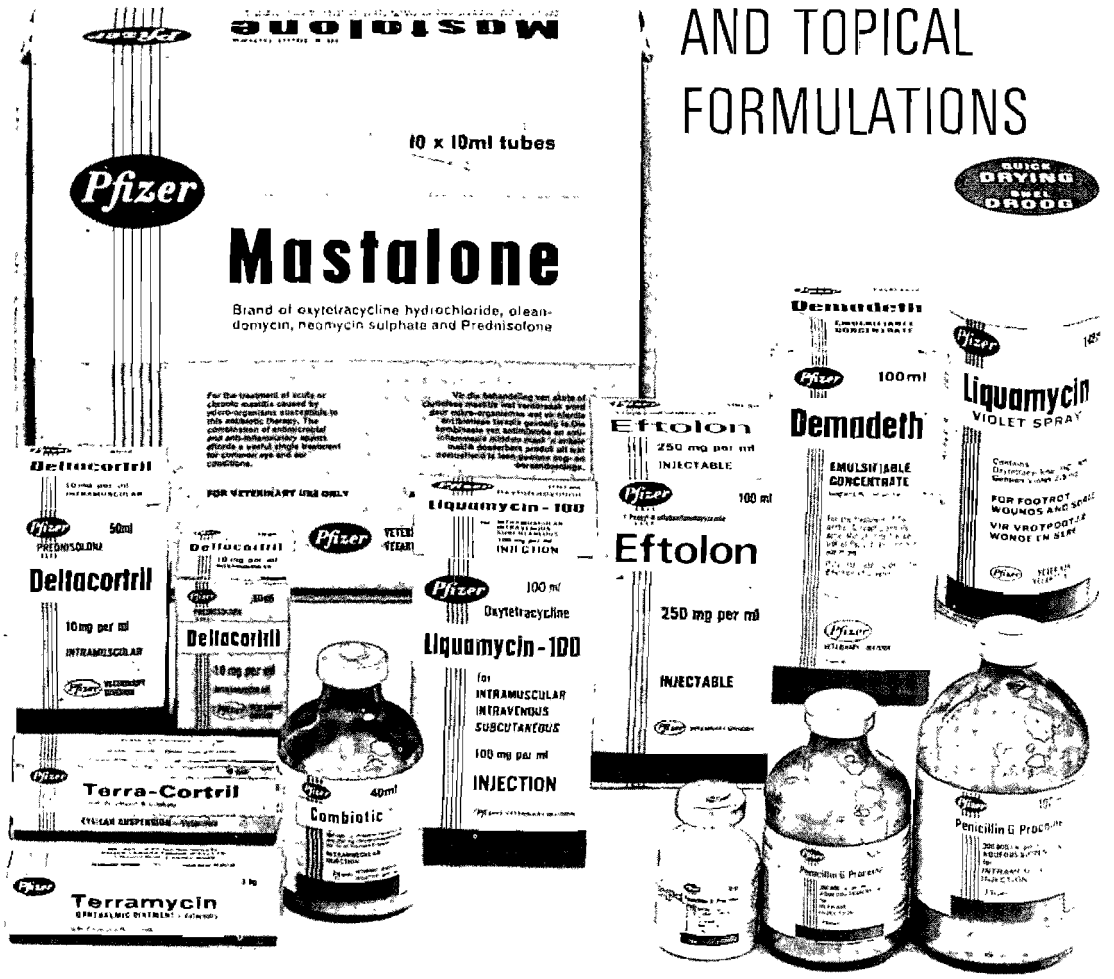


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# ON THE TOXICITY OF PROCAINE FOR PIGS

G. MITCHELL AND J.J.A. HEFFRON


## SUMMARY

Procaine caused respiratory arrest in halothane-anaesthetized normal Landrace pigs at a dose of 4,2 - 8,2 mg/kg. In some cases death due to respiratory failure occurred after giving 10 - 13 mg/kg. Because of its toxicity in pigs procaine is unsuitable for treating the porcine malignant hyperthermia syndrome.


## INTRODUCTION

In spite of its established toxicity[7] procaine is used as a specific treatment for the syndrome of malignant hyperthermia (MH) in both man[11] and the pig[5]. Its use as a therapeutic agent stems from its chance administration during an attack of the MH syndrome[1], from *in vitro* studies[10], and from studies in the pig[5 6]. The rationale for using procaine is based upon the finding of Feinstein[2] that local anaesthetics such as procaine block caffeine-induced contracture in frog skeletal muscle, and of Moulds and Denborough[10] that procaine markedly reduces halothane-potentiated caffeine-induced contracture in normal human muscle. While procaine is undoubtedly useful prophylactically in MH susceptible animals undergoing halothane anaesthesia[6], several reports indicate that procaine is ineffective in treating the established porcine MH syndrome[3 4 9]. MacLachlan and Forrest[8] have also reported a case in which procaine was ineffective in treating the human MH syndrome.

In our previous study[9] we suspected that respiratory failure was the preterminal event after procaine was given to reverse the halothane-induced MH syndrome in Landrace pigs. In this paper we have assessed the effect of the drug on respiration in normal Landrace pigs undergoing halothane anaesthesia since we were unable to find adequate data on the effect of procaine or its toxicity in pigs.

## PROCEDURE

Five German Landrace pigs were given an induction dose of thiopentone sodium ('Intraval', May Baker) after which anaesthesia was maintained with halothane ('Fluothane', I.C.I. Ltd., Johannesburg) in a closed circuit system, a 'Fluotec' Mk III vaporiser (Cyprane Ltd.), and a close-fitting face mask. The concentration of halothane used to maintain anaesthesia was 3-4% at an oxygen flow rate of 1,2 - 2 l/min. After approximately thirty minutes exposure to halothane, procaine (Procaine HCL, Propan Pharmaceuticals, Johannesburg) was introduced into the left jugular vein which had been previously exposed. Procaine was administered as a 2% solution in approximately 25 ml aliquots while respiratory movements were observed on the rebreathing bag.

Medical School; Univ. of Witwatersrand, Hospital St. Johannesburg 2001

## RESULTS

From Table 1 it can be seen that in all cases administration of procaine at a dose of 4,2 - 8,2 mg/kg body weight caused arrest of breathing. In each case this produced only a transient depression of respiration lasting between 1 and 3 minutes. However, in two of the five pigs a subsequent injection of procaine produced permanent respiratory depression while in the remaining three pigs respiratory movements restarted spontaneously and the pigs survived.

Table 1: EFFECT OF PROCAINE ON RESPIRATION IN FIVE ANAESTHETISED GERMAN LANDRACE PIGS.

| Pig No. | Body Mass (kg) | Dose of procaine inhibiting respiration (mg/kg) | Total dose of procaine (mg/kg) | Result |
|---|---|---|---|---|
| 1. | 95.5 | 5.3 | 10.5 | Died |
| 2. | 104,0 | 4,8 | 12.5 | Died |
| 3. | 108.0 | 4.6 | 9.3 | Survived |
| 4. | 118.8 | 4.2 | 8.4 | Survived |
| 5. | 97.5 | 8.2 | 14.4 | Survived |

## DISCUSSION

The results presented clearly show that an effect of procaine injection into normal pigs is respiratory arrest. The dose range at which reversible depression was observed was 4,2 - 8,2 mg/kg while in two of the animals death due to respiratory failure occurred between 10 - 13 mg/kg. Although doses of 8 - 15 mg/kg did not produce irreversible inhibition of respiration in all the pigs, it was clear that additional injections of procaine would have only enhanced the established respiratory depression in the survivors. These results are in accord with our previous observations on malignant hyperthermia susceptible animals of the same breed[9]. The failure of procaine to reverse the MH syndrome is perhaps not surprising primarily because the procaine concentrations required to reverse contracture *in vitro* are not attainable clinically[13]. The concentrations of procaine required to reduce caffeine-induced rigor ranged from 1,8 - 3,7 mM [2 12], while

halothane-induced contracture required 5 mM [10]. A concentration of 5 mM corresponds to a dose of 6.8 grams for a circulatory volume of 5 litres, yet Wikinski et al.,[13] found that five minutes after administration of 4 g of procaine the highest blood level attained was 0,41 mM. Assuming a linear response 6,8 g would produce a maximal blood concentration of 0,7 mM, a value some 3-7 times less than the concentration of procaine required for partial reversal of caffeine or halothane-induced muscle contracture. This dose is 4-7 times greater than the toxic concentration in pigs.

From these observations we suggest that procaine is unlikely to be of use in the treatment of malignant hyperthermia in pigs.

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# SPECIFIC SEROLOGICAL IDENTIFICATION OF OSTRICH MEAT AND MEAT PRODUCTS

L.W. VAN DEN HEEVER AND SANDRA MARAIS*


## SUMMARY

Using aqueous urea solution extracts of heated ostrich muscle as antigen for the production of precipitating rabbit anti-ostrich sera, it was possible to specifically identify raw, heated (70-95°C) and air dried-salted ostrich meat by means of gel immunodiffusion tests. The sera did not react with chicken, turkey or horse meat or with beef in any form.

The soluble proteins extracted from ostrich meat heated to temperatures of 70°C for 30 minutes appear to constitute at least two closely related antigenic determinants of which only one is thermostable at temperatures above 70°C.


## INTRODUCTION

Food legislation requires that "meat other than that of bovines, sheep, pigs and goats shall bear a label indicating its nature"; this also applies to any preparation or mixture of meat, processed meat and manufactured meat products[2].

The ostrich *Struthio camelus* Linn. is indigenous to Southern Africa and it's domestication has led to commercialisation and the production of meat for human consumption. Apart from conventional cooking of fresh meat a considerable proportion is converted into biltong by salting and air drying the raw or partially heated meat.

Positive identification of several large consignments of suspect ostrich biltong became necessary for forensic purposes. Efforts to do so by the use of aqueous extracts of the biltong against commercial anti-avian sera were unsuccessful (Brig. L. Neethling, S.A. Police Labs., personal communication.) It was suspected that the meat had been heated before salting and drying.

Serological identification of raw food animal meats is well known and frequently employed, and reports have also appeared concerning the identification of biltong[3 6 7]. Serum was traditionally used as antigen for antiserum production, with aqueous extracts of homologous meat providing sufficient serum proteins for a visible homologous precipitin reaction. For heat processed meat this method was less than satisfactory, i.a. because of the heat lability and the low concentration of serum proteins obtained by simple aqueous extraction of muscle.

Sinell and Mentz reported that high concentrations of sarcoplasmic proteins, as determined by the Biuret method, could be obtained by the use of 6 M urea solutions to extract both raw and heat processed meats[5]. The urea was found not to alter the relevant characteristics of native material nor to affect species specificity. Such urea extracts were eminently suitable for use as antigens in the production of antisera which reacted well with urea extracts of both native and heated meat (up to 120°C).There was, however, a clear difference between the electrical mobility of the heated and native homologous material, the former being a highly thermostable protein[5]. These findings have led to important modifications of earlier serological methods for species identification of meat.

With the exception of a single reference to the failure of extracts of ostrich biltong to react positively with precipitating sera produced by immunisation of rabbits with bovine serum[7], no other reports on ostrich meat identification have appeared.

Positive identification of raw, heated and air dried salted ostrich meat (biltong), and differentiation between ostrich meat and that of bovines, equines, chickens and turkeys is the subject of this report.

## MATERIAL & METHODS

1 Extracts of muscular tissue were prepared according to the method described by Sinell and Mentz[5]; this included mincing, homogenisation in dry ice, extraction in 6 M urea solution, lyophilisation and reconstitution for use as antigen for antiserum production and as antigen for gel diffusion tests. Extracts were similarly made from minced meats heated for 30 minutes to 70°, 80°, 90°and 95°C, from biltong prepared from raw and heated beef, horse meat and ostrich meat, and from the biltong of unknown derivation. The protein content of such extracts was determined by means of the Biuret reaction method and found to fall within the 400-800 ug N/ml recommended by H.J. Sinell (personal communication.)

2 Antisera were produced by injection of rabbits with urea extracts of various meats, with and without Freund's complete adjuvant, using a system advocated by Sinell & Mentz[5]. Intraperitoneal booster injections of 3 ml of antigen were given subsequently on three occasions at weekly intervals. The resulting antisera gave clear and well defined precipitin reactions against autologous and homologous meat extracts in gel diffusion plates.

3 Ouchterlony double diffusion agar gel plates of 1 mm thickness were prepared immediately before use from 1% solutions of Special Noble agar (Difco) in phosphate buffer. Wells of 3 mm diameter were set into the gel by means of a standard punch at equal distances around a central well. After filling the wells with antigen or antiserum the plates were incubated at 27°C for 24 h before being processed and read.

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4 Electrophoresis plates were prepared on standard microscope objective glass slides, each having a 6,5 mm central longitudinal trough situated equidistant between two 1 mm diameter antigen wells. After filling the wells the plates were placed in an electric field of 250v/50 mA for 1,5 h and therafter 0,04 ml of antiserum was placed in the trough for final diffusion at 27°C for 20 h as described by Sinell and Kluge - Wilm[4].

## RESULTS AND DISCUSSION

1 On the Ouchterlony double diffusion agar plate a thick apparently single and a thinner precipitin line developed when testing rabbit anti-ostrich meat serum against the homologous meat extract and against an extract of biltong made from ostrich meat. No lines became visible when testing this antiserum against heterologous meats, i.e. that of equines, bovines, turkey and chicken.

2 On immunoelectro-osmophoresis two distinctly separate precipitin lines could be demonstrated when testing rabbit anti-heated ostrich meat serum (RAHOS) against the homologous meat extract; no lines developed against heterologous meats (See Fig. 1). According to Clausen[1] the double-humped arcs indicate the presence of an antigen with two main electro-phoretic mobilities but possessing identical or partially identical antigenic properties.


*Fig. 1*
Agar gel diffusion, by electrophoresis, of rabbit anti-heated ostrich meat serum (RAHOS) against heated ostrich meat extract (HOME) and heated beef extract (HBE).

3 Testing RAHOS prepared from meat heated to 70°C for 30 minutes against urea extracts of raw ostrich meat (ROME) and ostrich meat heated to 70°, 80°, 90°and 95°C as well as against a urea extract of the unknown biltong, clear precipitin lines developed in all instances (See Fig. 2)

Repetition of this procedure using RABS failed to elicit any visible lines.

From this it was firstly concluded that the unknown biltong was in fact derived from ostrich meat. From the similarity of the lines opposite the unknown biltong well and those opposite the well containing an extract of ostrich meat heated to 70°C it is also concluded that the biltong was made from meat heated to a similar temperature. From Fig. 2 it can be seen that lines opposite ostrich meat heated to a higher temperature were single and less distinct. The spur formation at the point of contact between the lines of precipation opposite the wells containing extracts of meat heated to 70°C and those heated to temperatures above 70°probably results from reaction of the RAHOS with a multiple antigen or an antigen possessing different determinant groups[1]. It would therefore seem that the antigen produced by extraction of water soluble proteins from meat heated to temperatures above 70°C is a single relatively heat stable protein; conversely, extracts of meat which is raw or heated to temperatures below 70°C appear to contain at least three and two antigens respectively, one of which is labile to heat above 70°C.

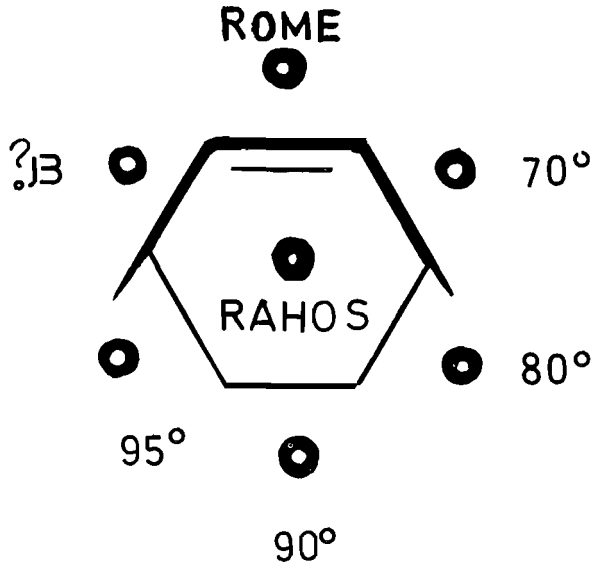


*Fig. 2*
Representation of double agar gel diffusion test of urea extracts of unknown biltong (?B) and or raw (ROME) and heated (70° - 95°C) ostrich meat against rabbit anti-heated ostrich meat serum (RAHOS)

In contradistinction to Sinell and Mentz[5] it was found that clearly defined precipitin lines were visible in the homologous systems when using the Ouchterlony double diffusion gel method. The duplicity of the antigen determinants resulting from urea extraction of meat on which they report was however confirmed in our studies as was the thermostability of one of the antigen components.

In the double diffusion tests in which RAOS was set up against extracts of raw and heated chicken and turkey meat, no visible precipitin lines could be detected by use of the technique employed. From this is it is concluded that our anti-ostrich serum did not produce cross-reacting precipitin lines with chicken and turkey meat extracts and that it can be employed for distinction between these two avian species and ostrich meat.

In a double diffusion test using rabbit antibeef serum (RABS) centrally and extracts of known beef biltong and the unknown biltong peripherally, a clear precipitin line developed in the homologous system. No precipitin lines were visible opposite the unknown biltong, and this further indicates that the unknown biltong was not derived from beef.

## CONCLUSIONS

From the above it is concluded that specific precipitating rabbit antisera can be produced against raw and heated ostrich meat which will positively identify such ostrich meat and also distinguish it from equine, bovine, chicken and turkey meat, whether raw or heated up to 95°C.

ACKNOWLEDGEMENTS

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# BOOK REVIEW BOEKRESENSIE

## THE VETERINARY ANNUAL

EDITED BY C.S.G. GRUNSELL AND F.W.G. HILL. FIFTEENTH ISSUE.

Wright-Scientechnica, Bristol 1975.

pp xviii +483. Figs 120 (4 colour). Tabs 58.

The enlarged fifteenth edition of this well known work is contributed to by no less than 87 distinguished authors and covers most fields of modern veterinary medicine. The clinician is well catered for with succint papers on numerous aspects of surgery, medicine, diagnostics and therapy of both farm and small animals. A review of fish diseases of 19 pages is beautifully illustrated with 16 photographs.

It is impossible to list the complete contents of this excellent book, but for interest's sake the following subjects are mentioned: the feeding of stored colostrum to calves, bovine respiratory disease, the treatment of bovine vaginal prolapse, "ringwomb" in sheep, intestinal haemorrhage in the pig, foal mortality, prostaglandins in equine stud management, diarrhoea associated with tetracycline therapy in horses, surgery of the canine hock, transfixation bolts in orthopaedic surgery, anaesthesia in very small cats and dogs, treatment of cardiac disease in the dog, myasthenia gravis, common poisonings and their diagnosis and treatment in small animals, canine renal punch biopsy, a review of mycoplasmal diseases in domestic animals, recent trends in veterinary therapeutics and the use of a computor in clinical research.

In addition to the subjects listed above general review articles on animal husbandry, reproduction and infertility, helminthology, anthelmintics and hypoxia, shock and healing are included.

The up-to-date and diverse information so ably presented in this latest issue of the *Annual* make it a worthy successor to the previous editions and a valuable source of continuing education in these busy times.

R.K.L.

# BOOK REVIEW BOEKRESENSIE

## THE USE OF MERCURY AND ALTERNATIVE COMPOUNDS AS SEED DRESSINGS

WORLD HEALTH ORGANIZATION TECHNICAL REPORT SERIES NO. 555, GENEVA, 1974.

pp. 29. Tabs. 6 Publ. price Sw. f. 5.-

Cereal seed must be treated with fungicidal dressing to prevent seedborne infection and to protect germinating seeds from soilborne pathogens. The organomercury compounds, being cheap and having a wide antifungal spectrum, are particularly suitable for this purpose. Such seed is, however, very dangerous if, for various reasons, it should be used as food or feed.

An interesting, brief review of the problem is given in this booklet and the relative dangers of the alkyl-, alkoxyalkyl and arylmercury compounds as well as hexachlorobenzene are discussed. The exceptional danger of alkylmercury compounds is pertinently emphasized.

Recommendations are made on the safe use of these products until suitably effective and cheap replacements have been developed.

T.W.N.

Jl S.Afr. vet. Ass. 46(3) 265-267 1975

# TRIALS WITH RAFOXANIDE*

## 7. EFFICACY AGAINST *FASCIOLA HEPATICA*, *HAEMONCHUS PLACEI* AND *BUNOSTOMUM PHLEBOTOMUM* IN CATTLE

A.J. SNIJDERS† AND I.G. HORAK††

SUMMARY

Anthelmintic efficacy studies involving 49 cattle either naturally or artificially infested with *Fasciola hepatica* are described. Some of the animals were also artificially infested with *Haemonchus placei* and *Bunostomum phlebotomum*.

At dosage rates of 3,75 and 5,0 mg/kg live mass rafoxanide was 64,6 and 92,6 per cent effective respectively against adult naturally acquired infestations of *F. hepatica*.

At 7,5 and 10,0 mg/kg it was 37,9 and 55,7 per cent effective against 42 day old liver fluke.

At 7,5 mg/kg it was 100, 87,4 and 94,6 per cent effective against mature *F. hepatica*, fourth stage *H.placei* and adult *B.phlebotomum* respectively.

## INTRODUCTION

The anthelmintic efficacy of rafoxanide (3'-chloro-4'-(p-chlorophenoxy)-3,5-diiodosalicylanilide) against *Fasciola hepatica* in sheep [1 2 4 6 10] and cattle [3 7 8] has been described by several authors. Its activity against *Fasciola gigantica* [13 14] and *Haemonchus placei* [13] in cattle has also been recorded.

The present paper describes anthelmintic efficacy trials against natural and artificial infestations of *F. hepatica* and artificial infestations of *H. placei* in cattle. Because of the excellent efficacy obtained against adult *Gaigeria pachyscelis* in sheep [6] it was decided to include *Bunostomum phlebotomum* in one of the experiments.

## GENERAL MATERIALS AND METHODS

A strain of *F.hepatica*, originally obtained from a naturally infested bovine has been maintained at our laboratory in sheep and *Lymnaea columella* since 1969. Metacercariae from these snails, harvested and counted on cellulose strips were used for artificial infestation of the cattle.

Some of the cattle were infested orally with the larvae of *H. placei* and percutaneously with those of *B.phlebotomum*, both of which have been maintained in pure culture in calves raised under worm-free conditions.

The rafoxanide was administered intra-ruminally as a 2,5% preformed suspension either by means of a trochar and cannula or by means of a stomach tube.

At autopsy the liver fluke and nematodes were recovered and counted by methods already described[6].

## *EXPERIMENT 1*

### *Materials and Methods*

Thirteen, nine-month-old, Africander type calves were each infested with 400 metacercariae of *F.hepatica* and allocated to three groups.

Forty-two days after infestation five calves were treated with rafoxanide at 7,5 mg/kg and five at 10,0 mg/kg live mass. All the calves were slaughtered 47 to 49 days after treatment.

### *Results*

The ranked fluke burdens are summarized in Table 1.

Table 1: EXPERIMENT 1: THE EFFICACY OF RAFOXANIDE AGAINST 42–DAY OLD *F. HEPATICA*

| | Numbers of *F. hepatica* recovered | |
|---|---|---|
| **Controls** | **Treated at 7,5 mg/kg** | **Treated at 10,0 mg/kg** |
| 171 | 22 | 4 |
| 177 | 40 | 32 |
| 208 | 157 | 65 |
| | 170 | 109 |
| | 188 | 202 |
| 185 | 115 | 82 |
| % Eff | 37,9 | 55,7 |

Rafoxanide at 7,5 and 10,0 mg/kg was 37,9 and 55,7% effective respectively against six-week-old *F.hepatica*.

### *Discussion*

Immature liver fluke in cattle are not as susceptible to the effects of rafoxanide as are fluke of the same age in sheep.

Presidente and Knapp[8]recorded a mean efficacy of

* RANIDE : Reg. Trade Mark of MSD (PTY) LTD, Merck Sharp & Dohme International, Division of Merck & Co., Inc., Rahway, N.J., U.S.A.

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58,8% against 42- to 47- day old *F. hepatica* in calves drenched at either 7,5 or 10,0 mg/kg live mass. In the present experiment these dosage levels resulted in efficacies of 37,9 or 55,7 per cent. In contrast a dosage level of 7,5 mg/kg resulted in a mean efficacy of 88,9% against 42 day old *F. hepatica* in sheep[6].

This difference in efficacy is probably due to the slower growth rate of *F.hepatica* in cattle when compared with that in sheep [12], thus resulting in smaller and less susceptible immature fluke in the former host species at the time of treatment.

## *EXPERIMENT 2*

### *Materials and Methods*

Seventeen, 18- month old Africander type cattle were selected on faecal examination from a herd naturally infested with *F.hepatica* and running in the Cradock district of the Cape Province.

Six of these animals were treated with rafoxanide at 3,75 mg/kg and six at 5,0 mg/kg live mass. The remaining five were slaughtered as untreated controls at the Cradock abattoir on the following day and the treated animals were transported to the laboratory where they were slaughtered 16 or 17 days later.

On counting, the liver fluke were divided into two populations consisting of individuals above or below 10 mm in length.

### *Results*

The numbers of *F.hepatica* recovered from the treated and control animals are summarized in Table 2.

Table 2: EXPERIMENT 2: THE EFFICACY OF RAFOXANIDE AGAINST A PATENT FIELD INFECTION OF *F. HEPATICA.*

| Bovine No. | Numbers of *F. hepatica* recovered | | |
|---|---|---|---|
| | < 10 mm | > 10 mm | Total |
| **Untreated controls** | | | |
| 36 | 24 | 240 | 264 |
| 48 | 76 | 921 | 997 |
| 186 | 51 | 353 | 404 |
| 187 | 56 | 353 | 409 |
| 195 | 59 | 438 | 497 |
| Mean | 53 | 461 | 514 |
| **Treated at 3,75 mg/kg** | | | |
| 37 | 23 | 287 | 310 |
| 41 | 5 | 62 | 67 |
| 45 | 24 | 292 | 316 |
| 50 | 1 | 89 | 90 |
| 188 | 100 | 198 | 298 |
| 194 | 7 | 2 | 9 |
| Mean | 27 | 155 | 182 |
| % Eff | 49,1 | 66,4 | 64,6 |
| **Treated at 5,0 mg/kg** | | | |
| 31 | 21 | 19 | 40 |
| 32 | 10 | 0 | 10 |
| 39 | 8 | 3 | 11 |
| 43 | 16 | 17 | 33 |
| 190 | 13 | 4 | 17 |
| 197 | 43 | 76 | 119 |
| Mean | 18 | 20 | 38 |
| % Eff | 66,0 | 95,7 | 92,6 |

At 3,75 mg/kg rafoxanide was 49,1% effective against fluke less than 10 mm in length and 66,4% effective against larger fluke. At 5,0 mg/kg these efficacies increased to 66,0 and 95,7% respectively.

### *Discussion*

The results obtained in this experiment are in marked contrast to those obtained against both natural and artificial infestations of *F.gigantica* in cattle[13] [14]. Rafoxanide at a dosage level of 2,5 mg/kg live mass resulted in efficacy of 98,4% against an adult natural infestation of *F. gigantica* [14] and a dose rate of 3,75 mg/kg in efficacy of 98,1% against 98-day old fluke [33].

## *EXPERIMENT 3*

### *Materials and Methods*

Nineteen, four-month-old Sussex x Africander bull calves were each infested with 400 metacercariae of *F. hepatica*. They were also infested percutaneously on a single occasion with 2930 infective larvae of *B.phlebotomum* and on 12 consecutive days with doses of 270 to 310 larvae of *H.placei* administered orally.

Eleven of the calves were treated with rafoxanide at 7,5 mg/kg live mass when the *F.hepatica* were 101 days old (mature), the *B.phlebotomum* 52 days old (adult worms) and the *H.placei* 3 to 14 days old (fourth stage worms). All the calves were slaughtered 21 to 24 days later.

### *Results*

The ranked worm burdens of the control and treated calves are summarized in Table 3.

Table 3: EXPERIMENT 3: THE EFFICACY OF RAFOXANIDE AGAINST ARTIFICIAL INFESTATIONS OF MATURE *F. HEPATICA,* FOURTH STAGE *H. PLACEI* AND ADULT *B. PHLEBOTOMUM.*

| Number of helminths recovered | | | | | |
|---|---|---|---|---|---|
| *F.hepatica* | | *H.placei* | | *B.phlebotomum* | |
| Controls | Treated | Controls | Treated | Controls | Treated |
| 23 | 0 | 365 | 25 | 4 | 0 |
| 33 | 0 | 810 | 31 | 7 | 0 |
| 34 | 0 | 1 051 | 44 | 8 | 0 |
| 56 | 0 | 1 087 | 83 | 31 | 0 |
| 58 | 0 | 1 122 | 104 | 34 | 0 |
| 77 | 0 | 1 246 | 146 | 42 | 0 |
| 88 | 0 | 1 292 | 154 | 75 | 1 |
| 88 | 0 | 1 591 | 166 | 93 | 2 |
| | 0 | | 200 | | 4 |
| | 0 | | 227 | | 5 |
| | 0 | | 309 | | 7 |
| 57 | 0 | 1 071 | 135 | 37 | 2 |
| % Eff | 100,0 | | 87,4 | | 94,6 |

Rafoxanide dosed at 7,5 mg/kg live mass was 100, 94,6 and 87,4% effective against adult *F.hepatica* and *B.phlebotomum* and fourth stage *H.placei* respectively.

*Discussion*

The numbers of *F.hepatica* and *B.phlebotomum* recovered from the untreated controls were disappointingly small when compared with the numbers used for infestation. The efficacy obtained against both these species, however, was so high that large worm burdens in the control animals were not required.

The efficacy against fourth stage *H.placei* is less than that obtained against fourth stage *H.contortus* in sheep[6], but whether this is a difference in host or parasite response cannot be determined from the present experiment. The results against adult *B.phlebotomum* closely resemble those against adult *G.pachyscelis* in sheep[6].

## GENERAL DISCUSSION

The above results further confirm the efficacy of rafoxanide against some of the important haematophagous parasites of sheep and cattle. Taken in conjunction with the effect against the larvae of *Oestrus ovis*[5 9] and *Gedoelstia hässleri* [11] they must lead to speculation as to the effect of this compound against other bloodsucking internal and external parasites.


## ACKNOWLEDGEMENTS

Mr. J.P. Louw, Mrs. Suzette Raymond and Miss Ina Penderis are thanked for their able technical assistance.

Dr. C.H.B. Marlow provided facilities for processing some of the autopsies in Experiment 2.


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## Bovine Leukemia - Cause Identified

After more than a decade of attempts by local and foreign scientists to determine the cause of bovine leukemia, American scientists have tentatively shown that a C-type virus is the causal agent. (Most viruses associated with other forms of leukemia are similarly classified by structure as C-type viruses).

The work, a co-operative project between the National Animal Disease Center, Ames (Iowa) and the University of Wisconsin, Madison, was facilitated by the use of a short-term lymphocyte technique developed by University researchers. Earlier attempts at identifying the agent responsible for this disease were hampered by difficulties experienced in establishing cell cultures from tumours or white blood cells of affected animals and the inability to link previously isolated viruses with bovine leukemia.

Further progress in this study will be greatly facilitated by a recently developed laboratory method for growing the virus in monolayer cell cultures of tissue from leukemic cattle. Large-scale concentration and purification of the virus will ensure a continuous supply of the virus, in contrast to the previously used lymphocyte culture technique.

Until the scientists can demonstrate tumour production by the candidate virus in lymph tissue of cattle, the above results are to be regarded as tentative. Thus far, studies have already shown the ability of this virus to produce tumours in sheep... a species in which leukemia rarely occurs.

"Agricultural Report" Washington D.C. Agricultural Counsellor (Scientific); Embassy of South Africa; February 1975

## STILLBIRTHS IN SWINE

It has been reported by sources in the U.S. that sows with blood hemoglobin of less than 9 mg/100 ml gave birth to more stillborn pigs than females showing normal hemoglobin readings. The injection of 500 mg of iron dextran or the addition of 100 ppm of iron sulphate to the ration prior to parturition reduces the herd stillbirth rate to less than 5%.

*Iowa State Veterinary Medical Extension Newsletter*, March 1975, No. 78.

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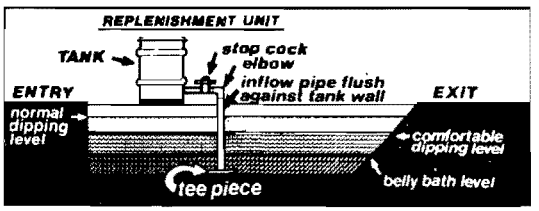


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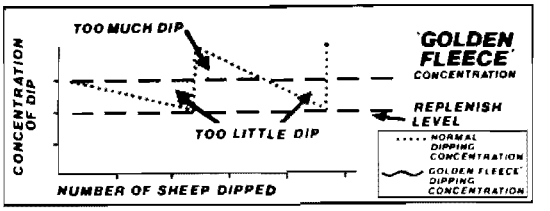


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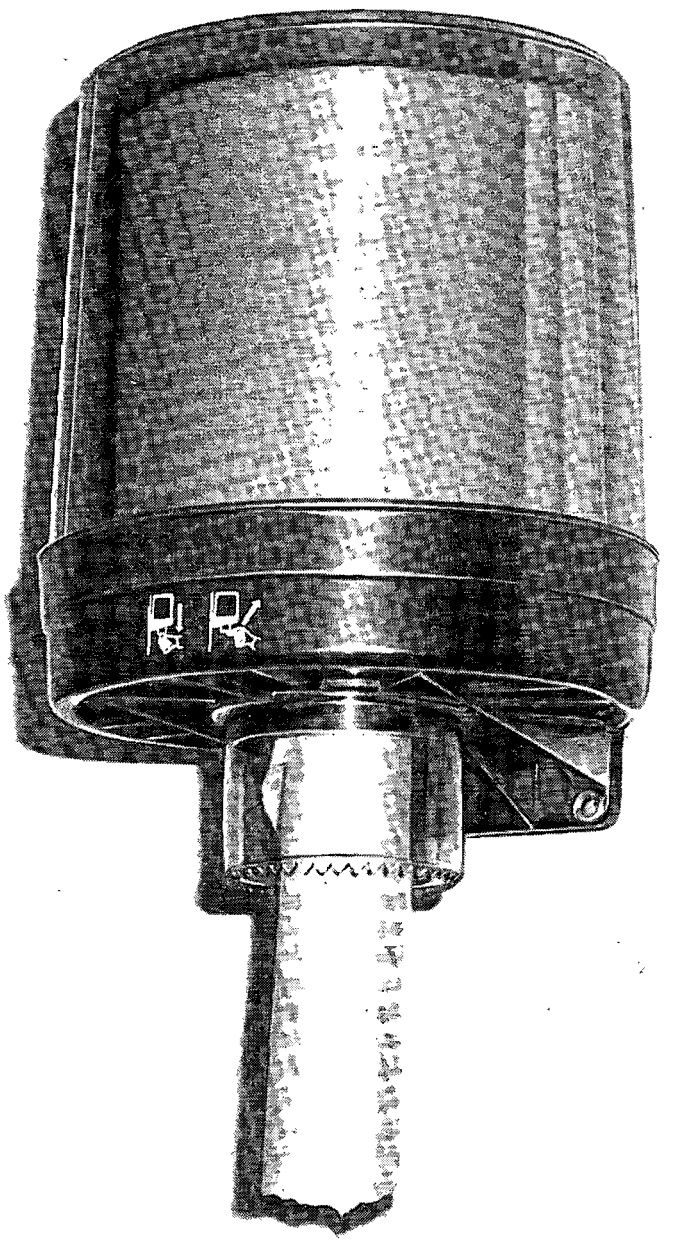

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Jl S.Afr. vet. Ass. 46(3) 271-272 1975

# THE EFFICACY OF CAMBENDAZOLE* AGAINST CESTODE INFESTATIONS IN LAMBS

I.G. HORAK● & A.J. SNIJDERS†


## SUMMARY

The anthelmintic efficacy of cambendazole dosed orally at 20 mg/kg live mass was determined against naturally acquired cestode infestations in lambs.

The anthelmintic was completely effective against both *Moniezia* spp. and *Avitellina centripunctata*.

Difficulties in ascertaining the presence of infestations with the latter species in the living animal are discussed.


## INTRODUCTION

The efficacy of cambendazole (isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate) against naturally acquired *Moniezia* spp. infestations in sheep has been reported in previous papers[1] [2]. In one of the experiments reported the anthelmintic appeared also to be effective against *Avitellina centripunctata*. The number of sheep treated and the proportion of controls infested with this cestode were, however, too small to allow valid conclusions to be drawn.

In a subsequent trial lambs which were infested with both *Moniezia* spp. and *A. centripunctata* were treated and these results are now recorded.

## MATERIALS AND METHODS

During January 1973, the faeces of 410 Merino lambs in the Vrede district of the Orange Free State were examined for the presence of cestode segments. Forty-eight lambs, all of which were excreting *Moniezia* spp. segments, were found and 40 of these were transported to our laboratory.

On arrival at the laboratory 21 of the lambs (Group I) were stabled and 19 (Group II) were put out to *Eragrostis curvula* pasture, previously ungrazed by livestock.

### *Group I*

Nineteen of the 21 stabled lambs were treated orally on arrival with cambendazole at 20 mg/kg live mass, the other two were kept as untreated controls. Faecal collecting bags were attached to all the lambs and on the following day their contents were examined for segments. The lambs were then put out to *Eragrostis* pasture and were slaughtered for cestode recovery five or six days after treatment.

### *Group II*

Sixteen of the 19 lambs were treated with cambendazole at 20 mg/kg live mass six days after their arrival, the remaining three were kept as untreated controls. One of the treated lambs, which was extremely weak, died within 12 hours of treatment. Subsequent procedures were similar to those for Group I lambs.

## RESULTS

The results are summarized in the table.

Table THE EFFICACY OF CAMBENDAZOLE AT 20 MG/KG LIVE MASS AGAINST *MONIEZIA* SPP. AND *A. CENTRIPUNCTATA*.

| No. of lambs | Treatment | Number with segments in collecting bags | Mean number of cestodes recovered | |
|---|---|---|---|---|
| | | | *Moniezia* spp. | *A. centripunctata* |
| *GROUP I* | | | | |
| 2 | Control | 2 | 4 | 16 |
| 19 | Treated | 19 | 0 | 0 |
| *GROUP II* | | | | |
| 3 | Control | 2 | 2 | 7 |
| 15* | Treated | 12 | 0 | 0 |

* One lamb died within 12 hours of treatment and is not included in the table.

All the lambs in Group I excreted cestode segments on the day following treatment whereas five of the 19 in Group II did not.

At slaughter both untreated controls in Group I were infested with *Moniezia* spp. and *A. centripunctata*, while in Group II two of the controls harboured both species and one was infested with *A. centripunctata* only. The sheep which died shortly after treatment had a single *A. centripunctata*.

Not one of the other treated sheep in either group was infested.

## DISCUSSION

Both at the time of selection of the trial sheep and on examination of the contents of the faecal collecting bags attached to the control sheep only *Moniezia* spp. segments were recovered. Microscopic examination of the faeces of these sheep after sugar flotation also revealed only *Moniezia* spp. eggs.


* *BONLAM : Reg. Trade Mark of MSD (PTY) LTD, Merck Sharp & Dohme International Division of Merck & Co., Inc., Rahway, N.J., U.S.A.*

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It would thus appear that it is extremely difficult to determine *A. centripunctata* infestation in the living animal and its presence in the present experiment was entirely fortuitous. The fact that all the control sheep were infested with this species indicates a high incidence of infestation in the flock as a whole and the efficacy of cambendazole against *Moniezia* spp. and *A. centripunctata* can safely be assumed from the results of the trial.

The sheep were put out to *Eragrostis* pasture at the laboratory in an attempt not to alter their diets too much from that on the natural pasture on which they had been been grazing and in this way prevent a spontaneous loss of cestodes. This nevertheless occurred in Group II although it appears as though only *Moniezia* spp. were affected. One of the controls and the sheep that died failed to excrete segments in their faecal collecting bags (thus indicating that their *Moniezia* spp. infestation had been lost), but both sheep were still infested with *A. centripunctata* at the time of slaughter or death. It therefore seems reasonable to assume that a similar interaction existed at the time of treatment in those treated sheep in Group II which also failed to excrete segments after treatment.

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## TRIPLE VACCINE AGAINST EQUINE ENCEPHALOMYELITIS

The U.S. Department of Agriculture has licensed the first three component vaccine to protect horses from eastern equine encephalomyelitis (EEE), western equine encephalomyelitis (WEE) and Venezuelan equine encephalomyelitis (VEE).

The new vaccine uses the killed viruses of the three strains to provide immunity to this serious disease complex of horses. Combination vaccines using killed virus were previously available only for EEE and WEE; immunity against VEE had been provided only by modified live virus (MLV) vaccines, which require special restrictions and extra safety precautions in their use.

Since the new vaccine uses only killed virus components, it can be administered safely without extra restrictions. The vaccine must be administered by deep intramuscular injection of two doses 21 to 28 days apart, both for initial vaccination and for annual re-vaccination.

The developer and licensee is Jensen-Salsbery Laboratories, a division of Richardson-Merrell Incorporated, Kansas City, Missouri. U.S.Department of Agriculture issuance of this licence required that the product meet standards of purity, safety, potency and effectiveness in compliance with the Virus-Serum-Toxin Act of 1913.

*NEWS*: (812-75); U.S. Department of Agriculture, Washington, D.C. 20250 March 1975

## Eggshell Wastes in Poultry Feed

Apart from being an excellent source of calcium (as shown in feeding trials by the University of Missouri and Southern Illinois University), the protein content of eggshell meal is considerably, especially in meals processed at lower temperatures.

From an industrial point of view, eggshell meal is estimated to be worth at least $40 per tonne, compared with existing soybean meal and ground limestone prices of $160-$180 and $20 per tonne, respectively.

Phosphated eggshell would be of even greater value as a feed ingredient, containing 5 per cent protein, 19 per cent phosphorus and 25 per cent calcium. This has been produced in the laboratory, by reacting equal parts of eggshell meal and concentrated phosphoric acid, and oven-drying the resulting material at 93°C. Results obtained in feeding trials (see Table) show clearly that phosphated eggshell is an excellent source of phosphorus and calcium in young turkeys. It would be quite suitable as a replacement for feed grade dicalcium and defluorinated phosphates, which currently cost $175 to $195 per tonne.

Compared with current prices for feed grade phosphates and soybean meal, phosphated eggshell should be worth at least $200 per tonne. As such, it is obviously well worth considering, despite the fact that specially adapted equipment, viz. a small rotary-type kiln, is required to convert dried eggshell meal into phosphated eggshell.

Table 1. COMPARATIVE EVALUATION OF PHOSPHATED EGGSHELL AND MONOSODIUM PHOSPHATE IN A FOUR-WEEK FEEDING TRIAL WITH STARTING TURKEYS.

| Item | Avg. body wt. at 4-weeks | Percent of Bone ash 4-weeks | Feed/gain 0--4 weeks | Relative value |
|---|---|---|---|---|
| Phos. eggshell | 590g(1.3 lb.) | 42.8 | 1.67 | 99.5 |
| MSP[1] | 591g(1.3 lb.) | 43.0 | 1.62 | 100.0 |

[1] MSP, mono-sodium phosphate, was the reference standard in this trial.

In fact, it has been suggested that with the high cost of protein and phosphates, and increasing transportation costs, the poultry and poultry feed industries can no longer "afford" the current eggshell disposal procedures.

"Agricultural Report" Washington D.C. Agricultural Counsellor (Scientific); Embassy of South Africa; February 1975

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# PERCUTANEOUS INFESTATION OF CALVES AND LAMBS WITH *OESOPHAGOSTOMUM* SPP.

H.M. GERBER*

SUMMARY

*Oesophagostomum radiatum* and *Oesophagostomum columbianum* became established in low numbers when 2 calves and 2 sheep respectively were exposed percutaneously to pure cultures of infective larvae of these worms.

A further sheep infested in the conjunctival sac became heavily infested but this may have occurred via the lachrimal duct.

As far as can be ascertained this is the first time that development of *O. columbianum* has been demonstrated after percutaneous exposure.

## INTRODUCTION

Reinecke [4] described percutaneous infestation as a means of obtaining a pure infestation of *Bunostomum phlebotomum* from a mixed culture of larvae. On a number of occasions, however, when a mixed culture of *B. phlebotomum* and *Oesophagostomum radiatum* was available in this laboratory, both species developed in calves infested percutaneously [1]. Indeed, after a few passages the *O. radiatum* predominated to the extent that the *B. phlebotomum* strain was lost [1].

The usual route of infestation of calves and sheep with *O. radiatum* and *Oesophagostomum columbianum* is *per os* [2]. While Mayhew [3] reported that *O. radiatum* was able to develop after percutaneous infestation when in mixed culture with *B. phlebotomum* larvae, the possibility that pure cultures of *O. radiatum* and *O. columbianum* are able to penetrate the skin of calves and sheep, has apparently not been investigated.

This paper describes carefully controlled tests to determine whether calves and lambs can respectively be infested with pure cultures of *O. radiatum* and *O. columbianum* by the percutaneous route.

## EXPERIMENTAL METHODS AND RESULTS

### *Larvae*

The larvae used originated from pure strains of *O. radiatum* and *O. columbianum* maintained by oral infestation in the laboratory.

The various cultures used for infestation were tested for purity by examining at least 200 infective larva ($L_3$) from each before infestation. The purity was confirmed by larval identification and necropsy after infestation of experimental animals.

### *Housing*

The calves were separated from their mothers at 1 day of age and were thereafter housed under form-free conditions [4]. The sheep were maintained under worm-free conditions from birth until used in these investigations.

### *Anthelmintics*

Four to 5 days before infestation the animals were drenched with broad spectrum anthelmintics** at twice the therapeutic dose levels.

### *Infestation*

The animals were infested (as described below) outside the buildings in which they were housed.

### *Autopsy*

With the exception of Sheep 4, the ingesta and mucosae of the small and large intestine of each animal were examined *in toto* at autopsy as described by Reinecke [4] and the worms identified according to Mönnig [2].

### *Calves*

Two Friesian bull calves were available for the investigation.

Calf 1. The animal was 7 months of age at the time of infestation. The hair between the shoulder-blades was clipped with scissors and 100 000 $L_3$ *O. radiatum* in 2 ml water slowly applied in a thin film so that the larval suspension did not run off. Thereafter the calf was held for 40 minutes before the clipped area and the forequarters were washed thoroughly by means of a strong jet of water and by hand.

Calf 2. When 5 days of age, the inner thighs were thoroughly washed, dried and 1 ml of water containing 25 000 infective larvae of *O. radiatum* pipetted onto each thigh and spread over the washed area by means of a spatula. The calf was held in a sitting position for 45 minutes to prevent it licking the infested area, which was then thoroughly washed with soap and hosed down for 4 to 5 minutes. Thereafter the animal was held in a standing position and its whole hindquarters thoroughly washed and dried. This calf was housed in a sheep pen which had never been inhabited by cattle.

The results are summarized in Table 1. From these data it can be seen that worm eggs were detected in the faeces of Calves 1 and 2 on Day +48 and Day +36 after infestation respectively. At autopsy only *O. radiatum* was recovered; 38 worms were found in Calf 1 and 193 in Calf 2.

* Veterinary Research Institute, Onderstepoort, 0110.

** The anthelmintics used were levamisole (Ripercol, Ethnor), parbendazole (Helmatac, Coopers) and thiabendasole (Thibenzole, MSD).

TABLE 1 : FAECAL WORM EGG COUNTS, LARVAL DIFFERENTIATIONS AND HELMINTH RECOVERY IN 2 CALVES PERCUTANEOUSLY INFESTED WITH *O. RADIATUM*

| Days after infestation | Calf 1 | | Calf 2 | |
|---|---|---|---|---|
| | Eggs/g of faeces (E p g) | *O, radiatum* ** larvae hatched | Eggs/g of faeces (E p g) | *O, radiatum*** larvae hatched |
| + 36 | Negative | Negative | Positive* | – |
| + 38 | – | – | 100 | – |
| + 41 | – | – | 50 | – |
| + 42 | – | – | Positive | < 200 |
| + 45 | – | – | 100 | > 200 |
| + 48 | Negative | 4 | 150 | – |
| + 52 | – | – | 100 | > 200 |
| + 56 | Negative | 111 | 100 | – |
| + 60 | – | – | 200 | > 200 |
| + 63 | Negative | 9 | 100 | > 200 |
| + 66 | – | – | 50 | > 200 |
| + 70 | Negative | 15 | – | – |
| + 77 | Negative | 6 | – | – |
| + 81 | – | – | 100 | – |
| + 84 | Negative | 9 | – | > 200 |
| + 90 | – | – | 150 | – |
| + 91 | – | – | Autopsy | |
| + 98 | 50 | – | | |
| + 112 | Autopsy | | | |
| Autopsy | 38 *O, radiatum* | | 193 *O, radiatum* | |

** Only *O, radiatum* larvae were recovered.

* Negative by standard egg count methods, but some eggs recovered on total flotation

*Sheep*

Four Dorper (Black Head Persian x Dorset Horn) wethers were used.

TABLE 2: INFESTATION AND WORM RECOVERY IN THREE SHEEP

| Sheep | E p g | | | No. of *O. columbianum* recovered at autopsy (Day + 72) |
|---|---|---|---|---|
| | Day + 37 | Day + 59 | Day + 60 | |
| 1 | Negative | 200 | 100 | 17 |
| 2 | Negative | Negative | Positive | 9 |
| 3 | 300 | 600 | 400 | 261 |

Sheep 1 and 2 were 16 months of age at the time of infestation.

Each was infested in the groin with 50 000 $L_3$ of *O. columbianum*. The techniques of cleansing and infestation were similar to those used in Calf 2.

Sheep 3 (17 months of age) was infested orally with 5 000 *O. columbianum* larvae from the same batch as above and served as a control for Sheep 1 and 2.

The results are summarized in Table 2. Faecal worm egg counts and subsequent slaughter confirmed that only *O. columbianum* was present.

Sheep 4 was 5 months of age when exposed for a period of 20 minutes by placing 20 000 $L_3$ of *O. columbianum* in 1 ml of water in the conjunctival sac formed by pulling the lower eyelid away from the eye.

Faeces were positive for eggs when examined 40 days after infestation (Table 3) and remained positive for 3 months. Before death from oesophagostomosis on Day +136, the egg count rose as high as 4 000 e.p.g. of faeces. Larval cultures contained only *Oesophagostomum* larvae. No worm counts were done.

TABLE 3: SHEEP 4 INFESTED VIA CONJUNCTIVAL SAC-FAECAL WORM EGG COUNTS AND LARVAL CULTURES.

| Days after infestation | E p g | *O, columbianum* larvae hatched |
|---|---|---|
| + 40 | 700 | – |
| + 41 | 1 000 | > 200 |
| + 49 | – | > 200 |
| + 53 | – | > 200 |
| + 57 | – | > 200 |
| + 76 | 1 400 | – |
| + 97 | 2 700 | – |
| + 119 | 4 000 | – |
| + 136 | Died from oesophagostomosis | |

## DISCUSSION

Mayhew [3] showed that *O. radiatum* in a mixed culture with *B. phlebotomum* is able to infest calvers percutaneously. He obtained similar results when calves were tied up in a stanchion to prevent oral access to the exposed area and when the skin and hair were cleansed without tying up the calf.

It appears unlikely, therefore, that larvae remain on the skin and hair after thorough cleansing. Hence the present investigation provides very strong evidence that *O. radiatum* in pure culture is likewise infective on percutaneous exposure.

In the case of *O. columbianum* very few worms developed and further experiments will be necessary to prove these findings conclusively. It must be noted, however, that the control sheep infested *per os* with 5 000 larvae also harboured very few worms and the larval viability may have been poor. Moreover, young lambs may have been more susceptible. On the other hand the ability to infest an animal percutaneously

may be a faculty of only a low percentage of these larvae from worms maintained by oral infestation and selection may result in greater viability after serial percutaneous passage. This aspect merits further investigation.

It is interesting to note that the sheep infested in the conjunctival sac with 20 000 *O. columbianum* larvae became so heavily infested that it died as a result of the worm infestation. Unfortunately it could not be ascertained whether any of the larvae penetrated the mucous membrane as all or some could possibly have passed down the lachrimal duct and nasal passages and then been ingested.

ACKNOWLEDGEMENTS

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## POULTRY DISEASES

At a recent meeting in the U.S.A. it was disclosed that the diseases of poultry are costing the industry approximately $200,000,000 per year. Many of these diseases are present in a chronic state and thereby lower weight gain, performance and feed efficiency without seriously jeopardizing the life of the bird. With the current increased feed cost and scarcity of feed ingredients, these disease conditions add considerably to the cost of a poultry operation. It was estimated that the U. S. industry spends about $30 million a year on coccidiostats, $40 million a year on antibacterials, $5 million for external parasite control, above $15 million for biologicals for viral infections and about $5 million for disinfectants. All of these are in addition to the unestimatable sum for vitamin and mineral additions to rations. In the U.S. leukosis, septicemia, air sac diseases and synovitis are major contributors to bird condemnation at time of slaughter. It is estimated that about 29 million broilers per year are condemned in the U.S. These birds would be worth $20 million.

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## DES AND CANCER

Much has been written and said about diethylstilbestrol residues in the tissues of animals and its relation to cancer. With calculations based on carcass information, it is estimated that 0.12 ppb of diethylstilbestrol is found in the liver and 1/10 of this level is found in the muscle of animals implanted with diethylstilbestrol. Annual average intake per human in the U.S. is 0.7 kg of beef liver and 0.5 kg of muscle meat daily. Thus, an average individual would intake a total of 0.007 mcg of diethylstil-bestrol daily. This would convert to a risk of one case of human cancer per 25 000 years in the U.S. population assuming that there is no threshold level for estrogenic hormones such as diethylstilbestrol. However, the human body, even in men, produces daily estrogens equivalent to 70 mcg of diethylstilbestrol. Consequently, it is inconceivable that 1/10 000 of this 70 mcg would produce a stimulus sufficient to cause human cancer.

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# THE PREPARATION, MINCING AND CANNING OF BOVINE OFFAL (RUMINAL AND INTESTINAL WALL) FOR HUMAN CONSUMPTION

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## SUMMARY

The results of a sudy of methods of cleaning and preserving bovine ruminal and intestinal wall by canning are described. Such offal could be converted into a hygienically satisfactory and safe food by laboratory washing and canning, and töe end product had a extended shelf life. Methods are described and suggested as a basis for ensuring safe utilisation of a valuable food product.


## INTRODUCTION

In terms of Act 87/1967[6], edible offal consists of *inter alia* the wall of bovine intestine and rumen. Although highly perishable, this product represents a major source of animal protein for the lower income groups of the population.

In the Republic some 15 000 and 3 000 tons of bovine and ovine/caprine ruminal offal are available annually for human consumption.

Due to the traditional preference shown by the conventional consumer, this material is sold as "rough" offal i.e. simply emptied of ingesta and roughly washed before issue for distribution. Such a product is highly contaminated and may pose a serious health hazard[4]. Obviously it does not meet the requirements of Act 36/1919[7] on clean, sound and wholesome food, free from disease, infection and contamination.

Methods to render this valuable animal protein safe for human consumption without prohibitive price increases to the conventional consumer are urgently needed. Horton and van den Heever[2] investigated chlorination, parboiling, curing or dehydration, used alone or in combination: parboiling for 5 minutes and cooling in brine for 1 - 3 hours renedered ruminal wall entirely free from coliforms, *E. coli, Staphylococcus* spp., and lactose negative microorganisms when cultured in selenite broth and on McConkey agar. They recommended parboiling and chilling in salt-nitrite curing solution for the elimination of vegetative microorganisms. and to achieve about 10% NaCl/Water phase for post treatment preservation of the product. Whereas raw washed bovine digestive tract offal had very high aerobic ($\bar{x}$ =651 x $10^6$; S.D. =661 x $10^6$) and anaerobic ($\bar{x}$ =33 x $10^6$; S.D. =58 x $10^6$) bacterial counts/g[2], the recommended treatment resulted in reduction of counts to some 50 x $10^3$ aerobic and 60 x $10^3$ of anaerobic bacteria/g of offal.

While such a process would bring about considerable improvement in the product, however, the fact that gastroenteritis is a socio-economic problem[5] makes it imperative that the product be safe for human consumption, and permit safe handling, storage and transport. Possibilities of recontamination and the consequent health hazard to consumer environment should be obviated and the keeping quality could be further enhanced. Enforcement of such requirements entail the consumer foregoing his traditional preference for raw rough offal still containing some ingesta. However, the nutritional and hygienic benefits derived from such processing could be justified and canning of minced offal was thus investigated.

Esty and Meyer[1] found that the hydrogen ion concentration had the least influence on the thermal death time of bacteria between pH 4, 5 and 11. They also found that table salt in 0,5 and 1% concentrations had a protective effect on spores when exposed to heat.

In the usual cooking of tripe for human consumption, table salt, and sometimes vinegar are added. Other acids which could be considered are citric and lactic acid.

## MATERIALS AND METHODS

Ruminal and intestinal tissue was derived from 59 slaughtered healthy bovines of various breed, weight, age and sex over an 8 month period.

### *Sampling:*

After slaughtering performed in terms of Act 87 of 1967[6] the rumen and intestines were opened, emptied of ingesta and rinsed under strongly running potable tapwater. Portions of 3 - 4 kg each, were removed from the digestive tract, aseptically cut into pieces of 10 $cm^2$ and mixed. A total of 83 aliquot samples thus collected were allotted at random to the various experimental series. (Table 1.)

### *Primary Processing:*

Primary processing of the specimens consisted of two washings which differed in their combinations of pH, temperature, time and additives. (See Table 1.) The unscraped specimens were processed within 60 minutes after slaughter in a rotary impellor type electric washing machine of 25 $\ell$ capacity.

The efficiency of primary processing was assessed by macroscopic inspection of the material for residual ingesta, and the general aesthetic appearance.

Subsequently the material was minced by means of a sterile mechanical mincer with extrusion sieve aper-

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x =Mean Value S.D. =Standard Deviation.

TABLE 1 : SCHEDULE OF SAMPLING AND PROCESSING OF OFFAL SPECIMENS FROM 59 BOVINES

| PRIMARY PROCESSING | | | | | | | SECONDARY PROCESSING | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | First Washing | | | Second Washing | | | | | | Additives | |
| Distribution of 83 sample aliquots | Processing Series No. | Additives & pH | Temperature (°C) | Time (minutes) | Additives & pH | Temperature (°C) | Time (minutes) | Distribution of sample aliquots | Processing Series No. | Processing Sub-Series No. | Number of Subdevided sample aliquots | Acid | NaCl | Sterilization time at 121°C (minutes) |
| | | | | | | | | 6 | | a | 36 | acetic | – | 60 |
| | | | | | | | | 5 | | b | 30 | acetic | – | 90 |
| 33 | I | Nil | 25 | 3 | Acetic acid | 67 | 3 | 5 | 1 | c | 30 | acetic | + | 90 |
| | | 7,2 | | | to | | | 7 | | d | 42 | – | – | 60 |
| | | | | | pH4 | | | 5 | | e | 30 | – | – | 90 |
| | | | | | | | | 5 | | f | 30 | – | + | 90 |
| | | Nil | | | Lactic acid | | | 10 | | a | 60 | lactic | – | 60 |
| 20 | II | 7,2 | 25 | 3 | to | 67 | 3 | 5 | 2 | b | 30 | lactic | – | 90 |
| | | | | | pH4 | | | 5 | | c | 30 | lactic | + | 90 |
| | | Nil | | | Citric acid | | | 10 | | a | 60 | citric | – | 60 |
| 20 | III | 7,2 | 25 | 3 | to | 67 | 3 | 5 | 3 | b | 30 | citric | – | 90 |
| | | | | | pH4 | | | 5 | | c | 30 | citric | + | 90 |
| 7 | IV | Nil 7,2 | 98 | 10 | – | – | – | 7 | 4 | a | 42 | – | – | 60 |
| 1 | V | Nil 7,2 | 25 | 3 | Potassium dihydrogen orthophosphate pH 6,9 | 67 | 3 | Discontinued | | | | | | |
| 2 | VI | Lactic acid to pH4 | 67 | 3 | Nil pH 7,2 | 25 | 3 | Discontinued | | | | | | |

tures of 3 mm diameter. The minced material of each aliquot was further sudivided into sub-aliquots (Table 1), and examined bacteriologically before sterilization.

*Secondary Processing:*

Secondary processing consisted of:

(i) Filling sterile glass canning jars of 360 g capacity with aliquots of the subdivided minced material of each batch.

(ii) adding 1 ml of acetic (99,5% w/w), lactic (88,5% w/w) or citric acid (1,0 g/ml aqueous solution) or 4 g of NaCl to the material according to schedule (Table 1) and thorough mixing with each additive;

(iii) light closure of the screw capped jars and heating in a conventional horizontal steam autoclave at temperatures and for periods of time indicated in Table 1;

(iv) tight closure of the screw caps of the hot jars immediately on opening the autoclave. The cooling off of the autoclave similar to the preheating time for sterilization, took about 30 minutes.

The samples subjected to combined treatments I - IV during primary processing (Table 1) were subjected to the corresponding treatments 1 - 4 of the secondary processing (Table 1).

*Laboratory Examinations:*

pH values were determined electrometrically.

Aliquots of 10g of material collected immediately after mincing but before administration of additives or sterilization were examined to assess the total number of viable aerobic bacteria present in the raw material by aseptic homogenisation in 90 ml of sterile buffer solution. Six tenfold serial dilutions in buffer were then made. Of each dilution 0,05 ml was dropped onto a blood tryptose agar plate. The plates were incubated aerobically for 12 hours at 37,5°C and the number of colony forming units (CFU) determined according to the method described by Miles & Misra[3].

After sterilization the jars were examined as follows:

(i) After incubation at 37,5°C for 10 days the batches of jars were subjected to visual examination for blowing and other signs of spoilage;

(ii) on termination of the incubation period, 4 jars of each batch were aseptically opened and from each sample 1 g of material was cultured in bromocresol-purple glucose tryptone (BcPGT) broth and incubated anaerobically at 37,5°C for ten days to permit regeneration of heat damaged and heat tolerant spores of anaerobic microorganisms;

(iii) the broth cultures were plated onto blood tryptose agar plates and anaerobically incubated for 10 days at 37,5°C;

(iv) Gram stained slides of any bacterial growth was examined microscopically;

(v) where any of the four jars were bacteriologically positive, the remaining unopened jars were discarded.

(vi) where all four jars of a batch were bacteriologically negative, two of the remaining jars

were heat shocked for 10 minutes at 80°C to encourage regeneration of any surviving spores. Samples from these were cultured in BcPGT broth and examined after 10 days and after 20 days incubation at 37,5°C.

To control the efficiency of the sterilization process five jars of heat processed canned offal (I.1.e. and I.1.f. Table 1.) were cooled to room temperature, and inoculated and mixed with 1 ml of a suspension of a broth culture containing:

140 000 organisms of *Clostridium oedematiens*
80 000 organisms of *C. welchii* type B
44 000 organisms of *C. welchii* type D
20 000 organisms of *C. botulinum* type C
80 000 organisms of *C. botulinum* type D

After incubation for 24 hours at 37,5°C these jars were then sterilized for 90 minutes at 121°C, incubated at 37,5°C for 20 days and examined bacteriologically as before.

The efficacy of the methods of reclamation of clostridia from specimens was tested as follows:

Each of the five clostridial culture suspensions were examined microscopically for bacterial spores.

One ml. of each clostridial culture suspension was inoculated into three 5 g samples of sterile minced tripe. After thorough mixing the containers were placed in a hot water bath at 60°C for 60 minutes. Subsequently 1 g of material from each of these was inoculated into two BcPGT broth tubes and incubated anaerobically for 10 days at 37,5°C and then plated onto blood tryptose agar plates for anaerobic incubation for 10 days at 37,5°C.

## RESULTS

*1. Primary processing*

Table 2: THE MACROSCOPIC APPEARANCE OF RUMINAL AND INTESTINAL MATERIAL AFTER PRIMARY PROCESSING.

| Experimen-Series (Table 1) | No. of Speci-mens | Status of Material | | Appearance |
|---|---|---|---|---|
| | | After Washing (Cleanliness) | After Mincing (Appearance) | |
| I | 33 | Clean | Smooth | Acceptable |
| II | 20 | Clean | Smooth | Acceptable |
| III | 20 | Clean | Smooth | Acceptable |
| IV | 7 | Not Clean | granular | Not acceptable |
| V | 1 | Not clean | Smooth | Not acceptable |
| VI | 2 | Not clean | Smooth | Not acceptable |

Primary processing according to the processes I - II (Table 1) produced aesthetically acceptable offal free from ingesta, and with an attractive appearance before and after mincing.

Offal processed according to the methods IV - VI (Table 1) were aesthetically unacceptable due to presence of ingesta, or were rendered less attractive by a granular and coagulated appearance.

Series V and VI (Table 1) of the primary processing were discontinued at this stage, as they offered no further potential advantages.

The bacterial contents and pH values of the unsterilized, minced material are indicated by the mean values ($\bar{x}$), standard deviations (SD) and coefficients of variance (CV) of Table 3.

Table 3: THE BACTERIAL STATUS OF MINCED RAW OFFAL AFTER PRIMARY PROCESSING.

| Series of Primary processing | No. of aliquots tested | Colony Forming Units/g | | | pH (Mean value) |
|---|---|---|---|---|---|
| | | Mean Value (x $10^6$) | Standard Diviation (x $10^6$) | Coefficient of Variance % | |
| I | 33 | 15 | 21,8 | 145,96 | 6,67 |
| II | 20 | 39,5 | 44,5 | 112,39 | 6,79 |
| III | 20 | 38,2 | 51,6 | 135,05 | 6,79 |
| IV | 7 | 20,5 | 41,6 | 20,9 | 7,04 |
| V | 1 | | Discontinued | | |
| VI | 2 | | Discontinued | | |

*2. Secondary processing*

Canning of the minced offal resulted in products of the microbiological status shown in Table 4.

A heating period of 60 minutes proved inadequate to sterilize any of the batches of canned offal, but no containers showed any gas formation during the 30 day period of incubation.

Heating for 90 minutes sterilized all of the experimental series of canned offal.

The unchanged physical appearance and quality of specimens, kept both at incubation and ambient temperature for 30 days indicated a shelf life of at least this period.

Reclamation of clostridial spores, using BcPGT broth for 10 days under anaerobic incubation at 37,5°C, followed by plating onto blood tryptose agar and 10 days anaerobic incubation at 37,5°C was effective for the recovery of all the clostridia tested.

## DISCUSSION

1. Offal material could be satisfactorily cleansed by washing for 3 minutes in tap water followed by acidified hot water (67°C). For the acidification of the hot water acetic, citric and lactic acid gave equally satisfactory results, and proved superior to potassium dihydrogen orthophosphate. Hot water used for the primary wash was unsatisfactory: 98°C for 10 minutes and 67°C for 3 minutes caused coagulation of the epithelium and adherence of the ingesta so that the product was not clean and of granular appearance.
2. Jars containing 360 g of offal were not sterile after 60 minutes heating at 121°C with 30 minute preheating and cooling periods. This situation was not improved by a primary wash at 98°C for 10 minutes nor by the addition of citric, acetic or lactic acid to the jars of minced material.
3. A 90 minute heat processing period was sufficient to sterilize all the batches of the experimental series, irrespective of additives.

## CONCLUSION

The results indicate that minced offal can be canned and effectively sterilized in glass containers. The sterilization procedure also eliminated artificially in-

Table 4: THE MICROBIOLOGICAL STATUS OF MINCED OFFAL AFTER DIFFERENT METHODS OF SECONDARY PROCESSING

| Primary processing (series) | DESCRIPTION OF SAMPLES Secondary processing series | subseries | Number of subdivided sample aliquots tested | MICROBIOLOGICAL RESULTS BACTERIOLOGY ($\times 10^3$CFU/g $\bar{X}$ | SD | CV(%) | MORPHOLOGY | pH $\bar{X}$ | SD | CV(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | a | 36 | 0.300 | 0,141 | 47,14 | cocci | 5,38 | 0,18 | 3,33 |
| | 1 | b | 30 | negative | negative | negative | negative | 5,46 | 0,05 | 1,00 |
| | 1 | c | 30 | negative | negative | negative | negative | 5,32 | 0,11 | 2,06 |
| I | 1 | d | 42 | 0,266 | 0,115 | 43,30 | cocci and bacilli | 7,00 | 0,24 | 3,49 |
| | 1 | e | 30 | negative | negative | negative | negative | 6,66 | 0,08 | 1,34 |
| | 1 | f | 30 | negative | negative | negative | negative | 6,50 | 0,0 | 0,0 |
| | 2 | a | 60 | 0,240 | 0,089 | 37,2 | cocci and bacilli | 5,80 | 0,46 | 8,00 |
| II | 2 | b | 30 | negative | negative | negative | negative | 5,88 | 0,10 | 1,86 |
| | 2 | c | 30 | negative | negative | negative | negative | 5,40 | 0,04 | 0,80 |
| | 3 | a | 60 | 0,440 | 0,260 | 59,2 | cocci and | 6,12 | 0,14 | 2,41 |
| III | 3 | b | 30 | negative | negative | negative | negative | 6,24 | 0,05 | 0,80 |
| | 3 | c | 30 | negative | negative | negative | negative | 5,50 | 0,0 | 0,0 |
| IV | 4 | a | 42 | 0,200 | 0,0 | 0,0 | cocci and bacilli | 6,67 | 0,56 | 8,52 |

troduced *Clostridium* spp. (Table 4). Thus the product was rendered safe for human consumption and the possibility of introducing new health hazards into the consumer's environment is eliminated.

The keeping quality of the offal product thus processed was excellent: the offal showed no change in quality, even after prolonged incubation, and a shelf life of at least 30 days was obtained.

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## Measuring Red Blood Fragility

A new diagnostic procedure for measuring red blood fragility has been developed by an Agricultural Research Service biological laboratory technician at the Veterinary Toxicology and Entomology Research Laboratory, College Station, Texan.

The new procedure requires only very small quantities of whole blood, and is about four times faster than the old method. Apart from its simplicity in technical operation, its rapidity and accuracy, the new method should be particularly useful in pediatrics and in small laboratory animals because of the very small amounts of whole blood required for diagnosis. It eliminates the need for using a needle and syringe (and the resultant psychological trauma experienced by children), as a pin-prick will yield sufficient blood for testing purposes.

Both the old and the new methods involve placing the red cells in solutions containing different amounts of sodium chloride to determine the degree to which cell membranes become permeable and lose haemoglobin to the salt solution (i.e. haemolysis). With the new method, however, the "waiting period" of several hours is greatly reduced.

It involves dropping 0,20ml of different salt concentrations into each of 12 tiny wells on a plastic titration plate, followed by 0,025ml of diluted whole blood in each of the wells. The plates are covered and incubated for 30 minutes at room temperature, given a brief centrifugation, and then examined for the points at which haemolysis begins and is complete.

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CLINICAL NOTE KLINIESE AANTEKENING

# BEEF CARCASE ICTERUS : AN EVALUATION OF DIAGNOSTIC METHODS IN TERMS OF VISUAL ASSESSMENT.

J.T.R. ROBINSON*

SUMMARY

Two laboratory tests on beef carcase fat, two on serum and one on urine from suspect icteric carcases were compared. Used singly on serum, the indirect van den Bergh and Fouchet tests were found to be most useful, and either of these two used together with the indirect van den Bergh test on fat gave results which agreed most closely with a subjective assessment of the marketability of suspect jaundice carcases after 24 hours' chilling.

## INTRODUCTION

The correct judgement of yellow beef carcases poses a meat inspection problems involving both diagnosis and evaluation. Yellow or yellowish discoloration may be the result of deposition of bilirubin in visible quantities in the tissues of the body and would be most obvious in the lighter coloured tissues. Alternatively it may result from accumulation of lipid-soluble plant pigments such as xanthophyll, the carotenes ($\infty$ $\beta$ and cryptoxanthin) and carotenoids in fatty tissues. The carotenes are known as provitamin A because they can be converted to Vitamin A in the small intestine and subsequently stored in the fat of the liver and elsewhere. Yellow fat may contain provitamin A and true vitamin A. Both are almost insoluble in water but soluble in alcohol and in ether. They are stable in the absence of $O_2$ and resist heat, acids and alkalis, but are easily destroyed by oxygen and light.

Degradation of haemoglobin to bilirubin takes place in the RE . system and is discharged into the plasma where it becomes bound to the plasma protein. Unless produced in amounts which are in excess of that which the liver can handle, the liver takes up the bilirubin and converts it into a water soluble pigment by conjugation with glocuronic acid and similar substances before discharging it into the bile. Carcase tissues may therefore be discoloured by fat soluble unconjugated bilirubin as well as water soluble conjugated bilirubin. Conversion of unconjugated bilirubin to, for example, a sodium salt, also renders it water soluble. Finally it should be pointed out that a carcase, derived from an animal which due to breed, age and/or nutritional status had yellow fat due to plant pigmentation, may simultaneously suffer from icterus.

It therefore becomes important to disthinguish between physiological discolouration due to plant pigments and the pathological deposition of bile pigments.

In a country such as Rhodesia where there is no convenient "conditionally passed" class into which many jaundiced carcases could be placed, a further distinction is desirable between the mild or recovering, cases where bile pigment is minimal and unlikely to affect the acceptability of the meat by the consumer, and the active severe cases where marketability is distinctly prejudiced by aesthetic considerations and the departure from a normal physiological state justifies condemnation.

A number of tests is advocated to aid the meat inspector in his diagnosis and judgement of yellow carcases. An investigation by the Bulawayo Meat Hygiene Laboratory sought to compare the results of some of these, and to correlate them with a final subjective visual assessment by experienced meat inspectors and veterinarians of the carcases after 24 hours' chilling; by this time the colour of the tissue had stabilised.

## MATERIALS AND METHODS

### BIOCHEMICAL TESTS

Over a period of approximately two years, a total of 159 beef carcases detained for suspected icterus was subjected to the following:

1. *The alkali/ether phase test* on fat which seeks to differentiate bilirubin from carotenes by the latter's exclusive solubility in fat which is thus extracted into the ether phase only.

The method comprises boiling approximately 2g fat in a test tube with approximately 10 ml of 5% aqueous solution of NaOH. The fluid is cooled and 5 ml ether added and shaken. On standing, the aqueous and ether phases separate, and the yellow colour should predominate in one phase or the other. Yellow ether and a clear colourless NaOH solution indicates the presence of fat soluble plant pigments of vitamin A; the reverse indicates the presence of water soluble bile pigments[2]

2. *Urine diazo test* (Ictotest)[1] by which urine is dropped on to a test tablet placed on test paper, and a violet ring indicates the presence of bile pigments in the urine.

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3. *Fouchet's test on serum:* Fouchet's reagent consists of 0,9% ferric chloride in 25% trichloracetic acid, and is commonly used as a urine test for urobilin. It was found that the mixing of 2 drops Fouchet's solution with a similar amount of clear serum on a white tile produced a coagulum which developed a distinct green tinge within 60 seconds if bile pigments were present in the serum[3].

4. *Modified indirect Hijmans van den Bergh diazo tests* on serum and fat[4].

*Method:* After mixing standard diazo (v d Bergh) A and B solutions in the ratio 10 : 0,3 to make diazo reagent, tests were as follows:

(a) The serum test: In the stated order the following are dispensed into a test tube and mixed: 2,8 ml distilled water, 0,4 ml serum, 0,8 ml diazo reagent, 4,0 ml methanol.
(b) The fat test: 20 g of fat is cut into small pieces, soaked in 40 ml of methanol and frequently stirred for 15 minutes. To 4 ml of the resultant extract, 1 ml of diazo reagent is added and mixed.

*Reading:* The mixtures are allowed to stand for 15 minutes. A pink or mauve colour during this time is scored as positive, irrespective of the intensity of colour or the rapidity of development.

*Sampling*: About 30g of fat free of fascia, muscle and blood was collected initially from three sites - perirenal, epicardial and subcutaneous; at an early stage subcutaneous fat from the shoulder region, being more copious than epicardial fat, and more intensely yellow than peri-renal deposits, was sampled instead.

Urine was collected from the bladder, but the uncertainty of recovering an adequate amount militated against the usefulness of this test as a routine procedure, even if the results had proved reliable.

Serum was obtained from heart blood decanted into a dry bottle or directly into a certrifuge tube at the pluck inspection table. A minimum of one hour was allowed for clotting before centrifugation. For Fouchet's test, standing of the blood for 1 - 2 hours nearly always yielded sufficent clear serum without centrifugation, so that this test could be performed on the slaughter floor if required.

VISUAL ASSESSMENT:

After 24 hours' chilling, each carcase was reassessed visually by two experienced meat inspectors or veterinarians; where the carcase was so discoloured as to render it unmarketable, it was condemned, and this judgement was the criterion against which the usefulness of the biochemical tests was measured.

The purpose of the trial was thus to test the reliability of biochemical methods in detecting a degree of icterus that would render the carcase unfit for sale after the colour had stabilised overnight.

Spleen smears were usually examined for blood parasites.

## RESULTS

The phase and urine tests were discontinued after an initial period, so that the tested carcases fell into two groups:-

Group A - 33 carcases on which all five tests were carried out wherever possible.

Group B - 159 carcases on which only the two van den Bergh (fat and serum) and the Fouchet serum tests were carried out.

Group A - 33 carcases: 22 condemned, 11 passed on visual assessment (V A)

Table 1: RESULTS OF 5 TESTS ON 22 CONDEMNED CARCASES

| Test | Number of Tests | Number of Positives | Number of Negatives | Incidence of false Negatives in terms of V.A. |
|---|---|---|---|---|
| Phase | 22 | 7 | 15 | 68% |
| Urine diazo | 12 | 4 | 8 | 67% |
| Van den Bergh fat | 22 | 8 | 14 | 64% |
| Van den Bergh serum | 22 | 13 | 9 | 41% |
| Fouchet serum | 22 | 13 | 9 | 41% |

Table 2: RESULTS OF FIVE TESTS ON 11 PASSED CARCASES.

| Test | Number of Tests | Number of Positives | Number of Negatives | Incidence of false Negatives in terms of V.A. |
|---|---|---|---|---|
| Phase | 11 | 0 | 11 | 0 |
| Urine diazo | 11 | 0 | 11 | 0 |
| Van den Bergh fat | 11 | 0 | 11 | 0 |
| Van den Bergh serum | 11 | 0 | 11 | 0 |
| Fouchet serum | 11 | 1 | 10 | 9% |

Group B - 159 carcases : 102 condemned, 57 passed on visual assessment (V A).

Table 3: RESULTS OF THREE TESTS ON 102 CONDEMNED CARCASES.

| Test | Number of Tests | Number of Positives | Number of Negatives | Incidence of false Negatives in terms of V.A. |
|---|---|---|---|---|
| Van den Bergh fat | 96 | 35 | 61 | 64% |
| Van den Bergh serum | 90 | 60 | 30 | 33% |
| Fouchet serum | 87 | 58 | 29 | 33% |

Table 4: RESULT OF THREE TESTS ON 57 PASSED CARCASES.

| Test | Number of Tests | Number of Positives | Number of Negatives | Incidence of false positives |
|---|---|---|---|---|
| Van den Bergh fat | 56 | 2 | 54 | 4% |
| Van den Bergh serum | 48 | 5 | 43 | 10% |
| Fouchet serum | 46 | 3 | 43 | 6% |

The reliability of combinations of more than one test was found in Group B to be better than any single result. The best combination was the van den Bergh fat test combined with either the Fouchet's serum or the van den Bergh serum test.

The results of the two serum tests correlated so closely that it is considered immaterial which is done, and it is unnecessary to do both. The reliability of a combined fat and serum test is illustrated in Table 5.

Table 5: RESULTS ON 135 CARCASES ON WHICH VAN DEN BERGH FAT TEST PLUS A SERUM TEST (VAN DEN BERGH OR FOUCHET'S) WERE DONE.

| Total condemned on V A | Both Tests Negative | False Negative |
|---|---|---|
| 88 | 14 | 18% |
| **Total Passed on V A** | **Both Tests Negative** | **False Positive** |
| 47 | 1 | 2% |

Spleen Smear Results:

Total number of smears examined: 132

Total number positive for blood parasites: 75 (anaplasmosis: 72, babesiases: 3)

Of the positive smears, 11 occurred in passed carcases.

## CONCLUSIONS AND DISCUSSIONS

1. None of the simple tests undertaken singly could be relied upon to correlate accurately with the delayed judgement of yellow carcases chilled overnight. However, the combination of a diazo test on fat with either a diazo or Fouchet's test on serum is a valuable aid to meat inspectors.

2. The establishment of a diagnosis to account for an icteric condition is of obvious value at meat inspection. In cattle slaughtered in Bulawayo, by far the most common cause of icterus is anaplasmosis, followed by babesiases. Obstructive or hepatogenic causes are rare.

3. Based on the above results, the following procedure has been found to work very satisfactorily in Bulawayo:-

(a) Any suspicious yellow carcase and its offals are detained, and samples consisting of subcutaneous fat, heartblood and a spleen smear are submitted to the laboratory.

(b) Van den Bergh and Fouchet's tests ared done on fat and serum respectively, and where both are positive, the carcase is condemned. Where one or both tests are negative, the carcase will be chilled overnight and judged visually the following day, unless the veterinarian responsible has adequate grounds for making an earlier decision.

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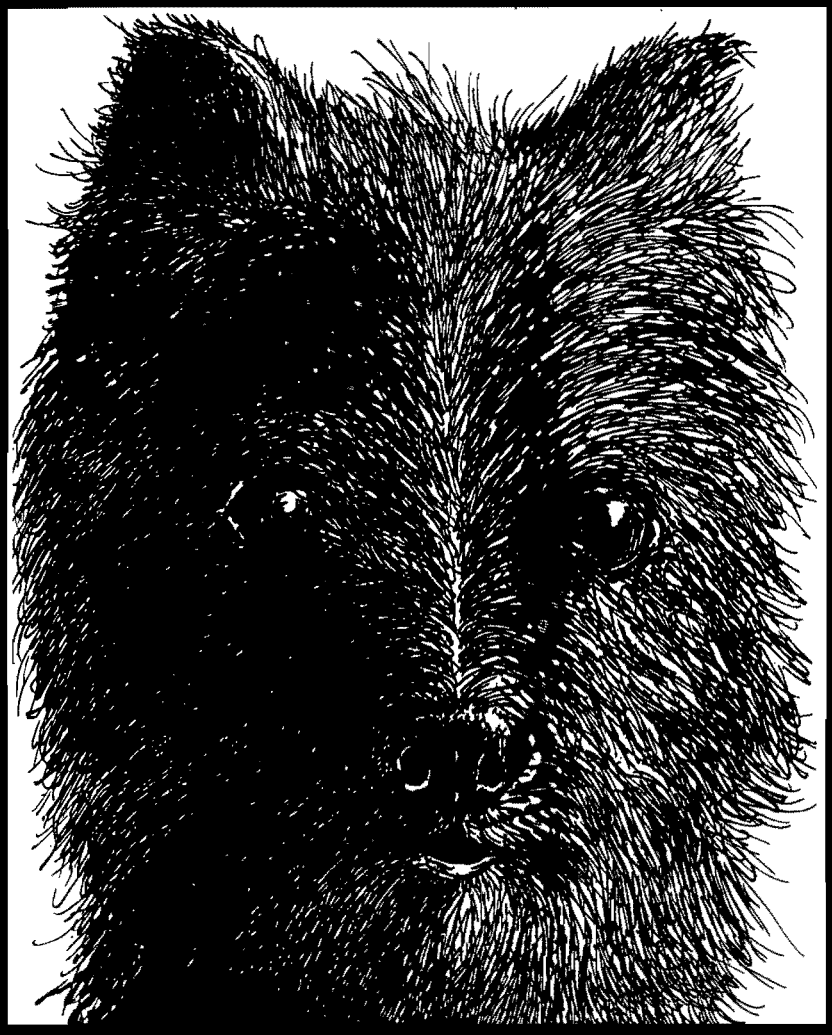

# LOPATOL

## The new broad spectrum Anthelmintic effective against both Roundworms and Cestodes in dogs

Lopatol, the new deworming product recently developed by Ciba-Geigy, has a broad spectrum of activity; specific for either nematodes or cestodes.

Nematodes – Ascarids: Toxocara canis, Toxascaris leonina
Hookworms: Ancylostoma caninum, Uncinaria stenocephala

Cestodes – Dipylidium caninum, Taenia pisiformis, Taenia hydatigena, Taenia ovis, Echinococcus granulosus

Safe and well tolerated by pregnant bitches and puppies, Lopatol which is orally administered, is particularly valuable in the treatment of ascarids which may be pre-natally contracted by puppies.

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Jl S.Afr. vet. Ass. 46(3) 285-286 1975

CLINICAL NOTE KLINIESE NOTA

# ECHINOCOCCOSIS (HYDATODOSIS) IN WILD ANIMALS OF THE KRUGER NATIONAL PARK

E. YOUNG*

SUMMARY

Echinococcosis has been diagnosed in the following wild species in the Kruger National Park : lion, *Panthera leo*, spotted hyena, *Crocuta crocuta*, Cape hunting dog, *Lycaon pictus*, Burchell's zebra, *Equus burchelli antiquorum*, buffalo, *Syncerus caffer*, hippopotamus, *Hippopotamus amphibius*, and impala, *Aepyceros melampus*. Infestation rates in the herbivores vary from 60% in zebra to less than 1% in impala. Species like elephant, *Loxodonta africana*, and blue wildebeest, *Connochaetes taurinus*, do not seem to be susceptible. The successful artificial transmission of *Echinococcus granulosus felidis* from Burchell's zebra to the lion is reported for the first time.

Hydatidosis has a country-wide distribution in South Africa and the average incidence is approximately 1% in all domestic species of slaughter stock [6]. In parts of the Transvaal Bushveld, however, the mean incidence exceeds 16 per cent[5].

Echinococcosis is also a common problem in various wild animal species. Five sub-species of *E.granulosus* are described from South African carnivores : *E.g. granulosus* (dog and other canidae), *E.g. africanus* (canidae; domestic and Cape wild cat, *Felis lybica), E.g. lycaontis* (Cape hunting dog), *E.g. ortleppi* (canidae, i.e. domestic dog and black-backed jackal, *Canis mesomelas*) and *E.g. felidis* (lion) [4].

In the Kruger National Park *Echinococcus* spp. have been recovered from spotted hyena and Cape hunting dog. In view of the fact that only small numbers of parasites have so far been found in hyenas, Verster (*pers. comm.*) does not yet consider the hyena as a true and suitable host of *Echinococcus*. It is suspected that these parasites might have been ingested with infested prey. *E.g. felidis* is a common parasite of lions in the Kruger Park. In the latter species exceptionally severe infestation is sometimes associated with diarrhoea, emaciation and debilitation, especially in young and very old lions.

*E.g. felidis* is host specific and is, like its definitive host, restricted to the Transvaal[4]. It has not been recorded from domestic livestock[5] and its intermediate host was previously unknown[6]. Recently, however, we have succeeded in repeatedly establishing heavy infestations of *E.g. felidis* in parasite-free lions by feeding them emulsified hydatid cyst material obtained from the livers of Burchell's zebra. Lions and zebra are found in significantly high numbers in the same parts of the Kruger National Park. Furthermore, the incidence of *Echinococcus* infestation in these zebra is 60 per cent. It therefore appears that Burchell's zebra may serve as an important natural intermediate host of *E.g. felidis*. A high incidence of hydatidosis in zebras has also been reported on by McCully *et al* (1969)[3].

Although Burchell's zebra and blue wildebeest are usually found together on the same pastures and both species are preyed upon quite heavily by lion, none of more than 300 blue wildebeest carcasses examined were infested with *Echinococcus*. More than 2000 elephant carcasses have been examined in the Kruger Park, but also with negative results. Both of these species seem to be resistant to natural infestation.

Hydatid cysts have also been found in buffalo (3-6% incidence) [1 7], hippopotamus (about 16% infestation rate)[2] and impala. Only three of 600 impala examined

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

were affected and this species also appears to be relatively insusceptible to hydatidosis[8].

In the intermediate herbivorous hosts the hydatid cysts are usually found in the livers and lungs. In zebra many large cysts (Fig.) often replace a considerable proportion of liver parenchyme and exert pressure on the remaining parenchymatous tissue. No other significant pathogenic effects on the intermediate hosts have so far been observed.

Undoubtedly further investigation will result in the recovery of *Echinococcus* spp. in other host species. Moreover, experimental infestations will provide more information on the susceptibility of primates and domestic livestock to the different sub-species of *Echinococcus* occurring in wild animals.

Although it has not been proved that contact with wild animals can result in hydatidosis in human beings one should always be aware of the seriousness of *Echinococcus* infestation and take the necessary precautions when handling wild carnivores.

ACKNOWLEDGEMENTS

I have pleasure in thanking:

1 The Veterinary Research Institute, Onderstepoort. the Division of Veterinary Services of the Department of Agricultural Technical Services and the National Parks Board for facilities and/or experimental animals.
2 Drs. A. Verster, P.A. Basson and D.V. Gradwell for reading the original manuscript and Dr. Verster for valuable advice and suggestions and the identification of parasites.
3 The Staff of the Veterinary Investigation Centre, Skukuza for technical assistance.
4 The Director of the Division of Veterinary Services for permission to publish.

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## The Dogbite Epidemic

According to *Newsweek*, March 24, 1975, dogbites have reached epidemic proportions in the United States. A 5-year-old boy was playing in his backyard when 3 dogs (2 collies and a Labrador retriever) loped by. The boy ran to the animals, and for a moment 1 of them licked his hand. Then, as the boy's mother watched from the kitchen window, the dogs attacked the boy in snarling rage, mauling and biting his head, neck and arms. After the dogs had fled, the mother rushed the boy to the hospital, but he died in the emergency room from shock and a loss of blood.

The boy was the victim of what public health officials regard as a major, but largely unrecognized, epidemic. For the period 1960-1970, the number of dogbites reported rose alarmingly in the United States. In St. Louis and Washington, D.C., the rate doubled. In Baltimore it went up 74% and in New York City 58%.

Six dogbite-associated deaths were reported to the Center for Disease Control in 1974. Health authorities estimate that about 1 million persons are bitten by dogs each year. The cost of treating dogbites runs to $50 million a year, and dogbites are rivaled by only gonorrhea for the top position among reported diseases and injuries.

Dr. Alan Beck of New York City's Bureau of Animal Affairs, who is convinced that statistics obtained from a study he conducted in St. Louis apply throughout the country, found that 60% of dogbites involve children under 15 yearso of age. Nearly 40% of preschool children are bitten on the head or neck, largely because they are at eye level with larger breeds. However, for all age groups, about 50% of dog bites are on the legs, 28% on arms and hands, and 13% on the body.

Although the last death in the U.S. caused by rabies from a dogbite occurred more than a decade ago, many victims still receive antirabies treatment, with its dangers of side effects.

Contrary to popular belief, only a small proportion of bites are inflicted by ownerless strays. More than 85% of dogbites involve privately owned dogs, and nearly half occur within 1 block of the owner's home. Dr. Beck theorizes that a dog's sense of territoriality plays a large part in the dogbite situation. The area in which a dog is habitually allowed to run comes to be regarded as its territory. Only 2% of bites are the result of deliberate provocation.

Health authorities think the dramatic increase in dogbites can be attributed to the growing popularity of large dogs, which are often purchased for protection in the large cities, rather than an increase in the dog population.

To curb the dogbite epidemic, Dr. Beck says owners should keep their dogs leashed or at home.

Source: "Veterinary Public Health Notes" U.S. Dept of Health, Education, and Welfare; Centre for Disease control March 1975

CLINICAL NOTE KLINIESE NOTA

# DYSTOCIA IN A FREE-ROAMING GIRAFFE *(GIRAFFA CAMELOPARDALIS)*

H. EBEDES*

A report that a giraffe cow (*Giraffa camelopardalis*) was unable to give birth was received in June 1972, in the Etosha National Park, S.W.A. Dystocia in free-roaming wild animals is not common and this was the first case recorded by the author after seven years in the Park.

The head, a portion of the neck and the right front leg of a full-term foetus was protruding from the cow's vulva. The cow was immobilized with a drug-mixture consisting of 3 mg etorphine hydrochloride (M-99, Reckitt), 80 mg phencyclidine hydrochloride (Sernylan Parenteral, Parke-Davis) and 20 mg trifluprómazine hydrochloride (Siquil, Squibbs Laboratory). Twenty minutes after darting she was unsteady and ataxic and was cast gently to the ground by means of a rope around her legs. She lay in sternal recumbency.

The cow was completely relaxed and after the rectal temperature was taken (104.5°F) a vaginal examination was performed. The allanto-chorion had ruptured with complete loss of amniotic fluids and the foetus was dry. No signs of straining were seen prior to darting. The foetus was in longitudinal, anterior presentation with flexion of the left shoulder and elbow. Because of the impaction of the lower neck and flexed shoulder in the pelvic canal, there was insufficient space for rotation of the foetus. The long length of the retained left limb and the short arms of the author also made it impossible to reach the carpal joint or metacarpus to attach a traction rope to correct the malpresentation as described by Benesch and Wright (1955). The possibility of caesarian section was considered, but because of the poor prognosis and lack of suitable housing facilities during the convalescent stage, it was decided to destroy the animal.

The post mortem examination confirmed the above findings. A large amount of purulent material had accumulated in the lower parts of the uterus and the uterine wall was oedematous.

An interesting finding in the foetus was malformation of the left carpal joint. The carpal joint could not flex at all and was overextended. This abnormality was probably the cause of the dystocia. During the expulsion phase of parturition the extremity of the left limb, due to the overextention of the carpus, was held back along the dorsum of the uterus and vagina and forced dorsally over the back of the foetus, preventing full extension of the limb. Simultaneously the neck of the foetus was expelled resulting in flexion of the shoulder and elbow joints.

ACKNOWLEDGEMENTS

The writer wishes to thank the Senior Nature Conservator of Etosha National Park, Mr. G. Visser and Nature Conservator J. Joubert for their assistance, Prof. J.S. van Heerden and Dr. A Schutte of the Faculty of Veterinary Science, Univ. of Pretoria for reading and commenting on the manuscript, and Mrs. H.D. Smith for typing.

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*PRIVATE BAG X5020 STELLENBOSCH 7600

# PREVENTION OF URINARY CALCULI IN CATTLE AND SHEEP

The exact cause of urinary calculi is still uncertain and quite complicated. This leads to uncertain preventative measures. The following suggestions seem to help dramatically in certain situations:

1. Sodium chloride can be used at the rate of 5% in a ration to increase water consumption.
2. Feed ammonium chloride at the rate of .75 to 1.5 oz. per head per day to cattle and .25 oz. per head per day to sheep. This changes the pH of the urine.
3. Analyze forage and rations, and balance the mineral content of the feed. This is particularly important with calcium phosphorus ratio as high phosphorus predisposes to the condition. Keep silica levels as low as possible. Provide adequate Vitamin A.
4. Keep intake of water high. This may require heating the water in the cold weather.
5. Keep stress at a minimum. Provide protection in cold weather, avoid rough handling, crowding, etc.

*Virginia Polytechnic Institute Veterinary Newsletter*, Virginia Polytechnic Institute State University, January 1974.

Jl S.Afr. vet. Ass. 46(3) 289-290 1975

CLINICAL NOTE KLINIESE AANTEKENING

# DYSTOCIA IN A FREE-LIVING IMPALA (*AEPYCEROS MELAMPUS*)

*V. DE VOS**

SUMMARY

Dystocia in a free-living impala (*Aepyceros melampus*) in Kruger National Park (KNP) is described. An exaggerated unilateral foot-nape posture is held responsible for a complete obstacle to parturition.

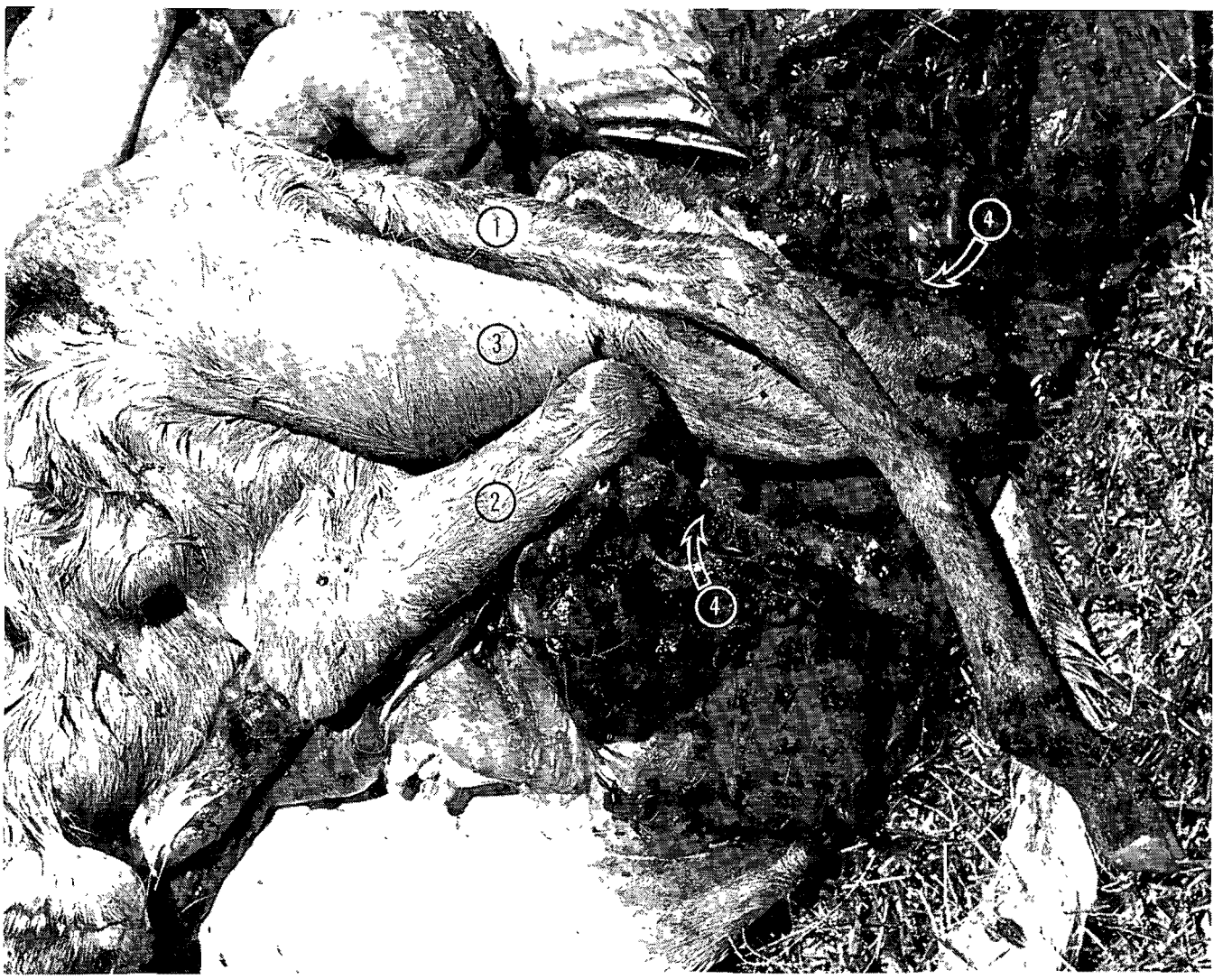


Fig.: Impala carcass opened to show the cause of dystocia. 1. Foetal left front leg in exaggerated foot-nape posture. 2. Foetal right front leg with flexion of elbow joint. 3. Neck of foetus. 4. Bony parts of maternal pelvic canal.

## INTRODUCTION

Dystocia implies some obstacle to parturition whereby the young cannot be delivered by maternal effort alone[1]. It is classified as maternal when the essential cause lies in the mother and foetal when the young one is primarily responsible. Although it is recognized as a common condition in domestic species the literature was found to be quite barren of reports on its occurence in wild animal species. The uniqueness of this condition in wild animals warrants this report of dystocia in a free-living impala.

*Department of Nature Conservation
National Parks Board
Skukuza

CASE HISTORY

An impala ewe with part of a foetal leg protruding from the vulva was observed near Skukuza Rest Camp in the KNP. The animal seemed nervous and was obviously in second stage labour. Intermittent straining sessions with forceful expulsive efforts took place; but to no avail. It was apparent that some obstacle was preventing the normal course of parturition. After a few hours, with no improvement and the ewe already in an exhausted state, it was decided to destroy the animal and determine the cause of dystocia.

EXAMINATION AND DISCUSSION

On opening the maternal carcass a full term foetus was found to be stuck in the pelvic outlet of the reproductive canal.

As depicted (Fig) the foetus was presented anteriorly with the left fore limb desplaced upwards and over the extended head. This is an exaggerated form of the unilateral "foot-nape posture" as described by Benesch and Wright[1]. This posture forced the head to the side of the pelvic canal and the neck onto the right shoulder, preventing extension of the right leg and caused further impaction of the unborn lamb.

Long-limbedness and a loose connection of the scapula are held as factors predisposing this condition in the horse. These anatomical features are even more pronounced in the impala lamb and must have some significance in the aetiology of the present case.

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# BOOK REVIEW

# RESENSIE

## EVALUATION OF SOME PESTICIDE RESIDUES IN FOOD

W.H.O. Pesticide Residue Series No. 2

World Health Organisation, Geneva, 1973

pp VI +587, Figs 21, Tabs. 122, Publ. Price Sw. fr. 25.00

This monograph series was prepared by pesticide experts of WHO and FAO who met in November 1972 and deals with information concerning a series of important compounds ranging from Azinphos-methyl to Thiabendazole. It contains information not available in earlier publications, but these should also be read for a complete evaluation of any given pesticide residue.

The compounds are listed in alphabetical order. Data is provided concerning name and synonyms, structural formula, properties, acceptable daily intake, biochemical aspects, toxicological studies and comments, food residues and evaluation, fate of residues, methods of residue analysis, tolerances, appraisal, recommendations, further data required and references. Annex 1 provides in tabular form a most useful index to documentation and a summary of recommendations concerning acceptable daily intakes, tolerances, practical residue limits and guideline levels as of November 1972.

This book provides the reader with authoritative and concise information on a subject of general importance to all and specific interest to many.

L.W. v.d. H

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## *MORE ABOUT STILLBIRTH IN SWINE*

*Dichlorvos, the well-known Shell anthelmintic, is a parasympathomimetic drug which increases the life of acetylcholine at the parasympathetic nerve endings by inactivating acetyl cholinesterase which results in stimulated muscle contraction. Feeding Dichlorvos during the last 21-30 days of the gestation period usually results in a decreased interval between pig births, a reduced stillbirth rate and an increased weaning rate. Neostigmine administered subcutaneously at 5 mg during parturition has also reduced the rate of stillbirths in swine.*

*Iowa State University Veterinary Medical Newsletter*, April 1975, No. 179

Jl S.Afr. vet. Asss. 46(3) 291-294 1975

CASE REPORT GEVALVERSLAG

# AORTIC STENOSIS IN A DOG

C. BUTTON*, G.D.IMES **, & V.A. LIEBMAN ***

SUMMARY

A case of valvular aortic stenosis in a dog is described. The presenting sign was syncope during exercise. An unusual feature was a right bundle branch block electrocardiographic pattern. During syncope electrocardiograms usually indicated atrial standstill and always had sever ST segment changes indicative of myocardial ischaemia. Pathological findings included chronic valcular changes which did not appear to be the result of a infectious inflammatory condition.

## HISTORY

The subject, an 8 month old male Alaskan Malamute, had been successfully treated for biliary fever (*Babesia canis* infection) at the age of three months.

At 6 months the dog began to faint during exercise. Preliminary examination revealed a systolic murmur and a bizarre QRS electrocardiographic pattern. The dog was treated with a digitalis preparation and rested. As there was no improvement and the dog was having up to 8 syncopial episodes daily, it was then referred to the Department of Medicine.

On enquiry the breeder of the dog reported that, to the best of his knowledge, neither parent of the patient nor any sibling nor ancestor had suffered from heart desease.

## CLINICAL EXAMINATION

The patient was very lively and hyperexcitable. He was well grown but poorly muscled and weighed only 27kg. Temperature was normal throughout. The resting pulse rate varied from 80 to 120 beats per minute. The pulse was "small" (of poor volume). The dog's excessive panting, possibly caused in part by hyperexcitability, rendered auscultation so difficult that detailed examination and phonocardiography had to be performed under general anaesthesia. The murmur was systolic, grade III/V (Detweiler and Patterson[4]), and was best heard over the left anterior thorax in the 2nd and 3rd intercostal spaces at the level of the costo-chondral junction. The murmur radiated widely and could be heard over the right anterior thorax and over the carotid arteries. The first heart sound, auscultated over the mitral area, was normal. There was no palpable precordial thrill, nor was there a ventricular heave. Mucous membranes were a normal pink colour. Appetite, water intake, urine and faeces were normal.

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## ELECTROCARDIOGRAPHIC EXAMINATION.

ECG's were recorded with the dog in right lateral recumbency. P Waves varied slightly in conformation, were slightly prolonged (0,05 sec) and often notched. The PR interval was 0,13 sec.

The QRS complex was prolonged (0,06 to 0,07 sec) and bizarre. Initial vectors were normal, but later vectors were aberrant; the QRS complex was represented by QRSR'S'R" on lead I. The amplitude of the waves was not exceptional (Fig. 1).

The QT interval was 0,12 to 0,13 sec. The ST segment showed elevation of up to 0,2mv on lead II. The T wave varied in amplitude from day to day, occasionally reaching +0,6mv on lead II.

On several occasions the dog was exercised until he fainted. He ran keenly with a handler; between one and 10 minutes of running precipitated syncope, except on one occasion, when sustained running did not result in syncope. Syncope was preceded by a sudden slowing down, a few staggering steps and a very relaxed fall. The dog's pupils dilated and its mucous membranes became moderately cyanotic. There was profuse salivation and breathing was deep but regular. Syncope lasted one to two minutes and recovery was preceded by panting or howling. The dog usually regained his feet within two and a half minutes of falling.

Occasionally the dog was lifted and connected to the ECG recorder within 30 seconds of falling. On the syncopial ECG the P wave was usually absent, there was a slight variation in the RR interval, and the heart rate was usually about 100 beats a minute. There was severe depression of the ST segment on leads I, II and III and aVF (maximum depression was 0,9mv on lead II) and elevation on leads aVR and aVL (Fig. 2). The ST changes gradually disappeared and P waves reappeared 60 to 90 seconds after recordings commenced.

## PHONOCARDIOGRAPHIC EXAMINATION

Phonocardiography was performed under general anaesthesia using a shortacting intravenous barbiturate*. The chest piece was placed over the point of maximum intensity of the murmur. The dog's nose

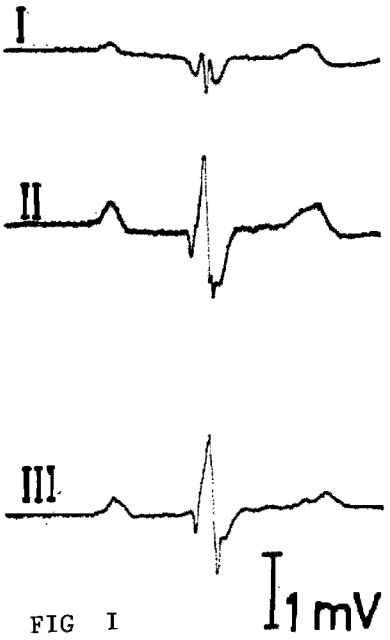


FIG 1

ECG leads I, II and III. Paper speed 100mm/sec.

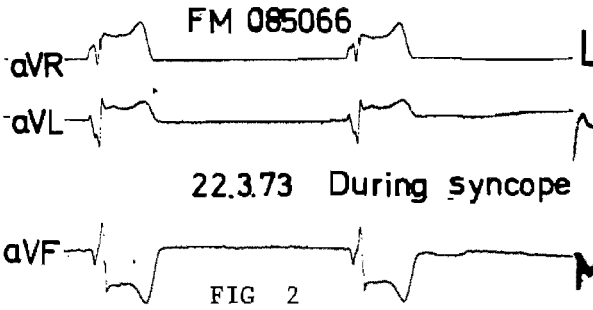


FIG 2

ECG leads aVR, aVL and aVF during syncope. ST segment changes are marked. Paper speed 100mm/sec.

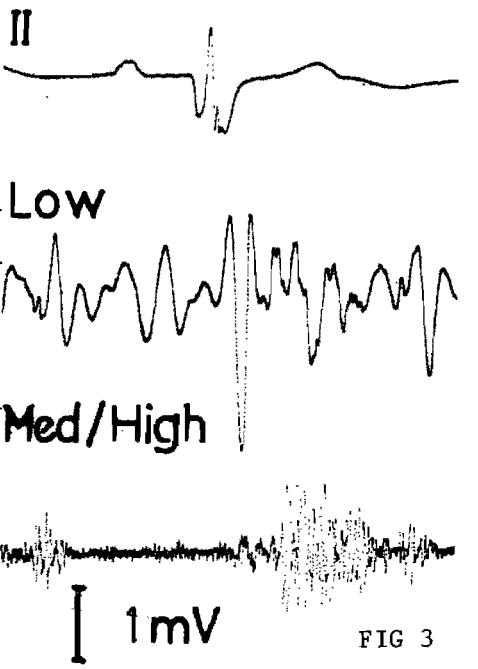


FIG 3

ECG lead II, low and medium/high frequency phonocardiogram. Paper speed 100mm/sec.

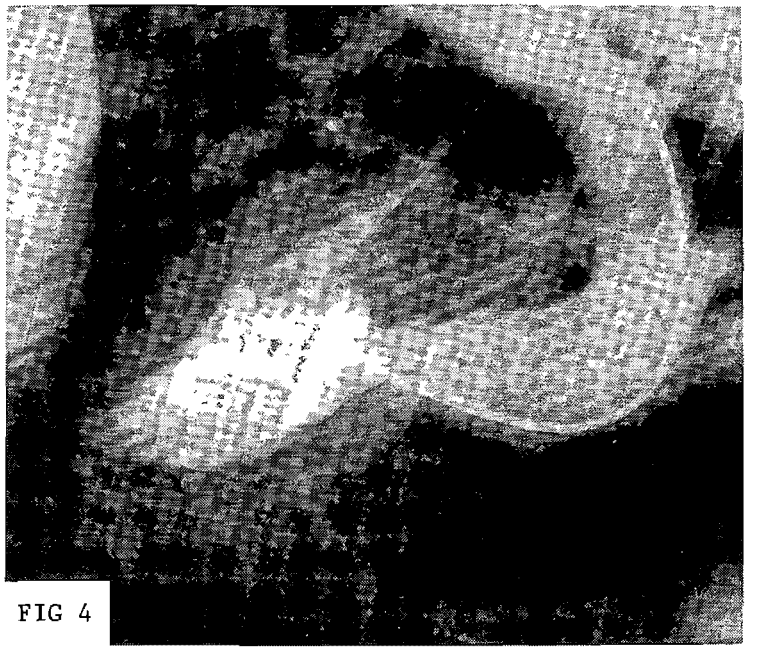

FIG 4

Angiogram showing the catheter in place in the left ventricle. The area of valvular stenosis and the post stenotic dilation of the aorta are easily seen.

FIG 5

Aortic semilunar valves. There is obvious thickening and loss of elasticity.

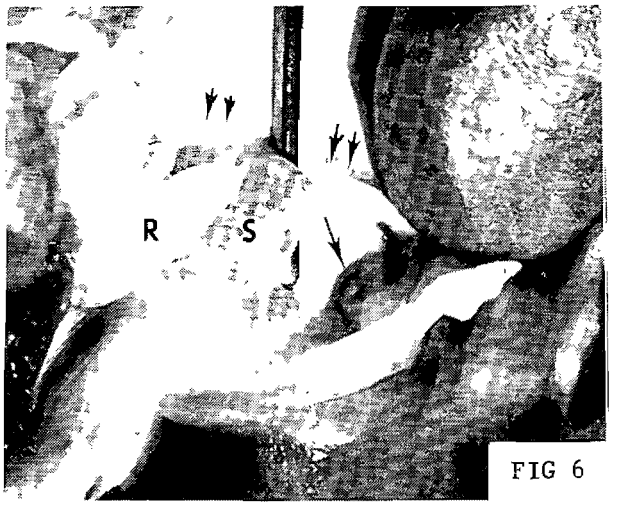


FIG 6

Right (R) and septal (S) semilunar valves. Note umbilicated nodule (arrow) on the wall of the sinus of Valsalva, myxomatous thickening of the leaflet (in front of probe) and projecting ring of fibrous tissue in the aorta (double arrows).

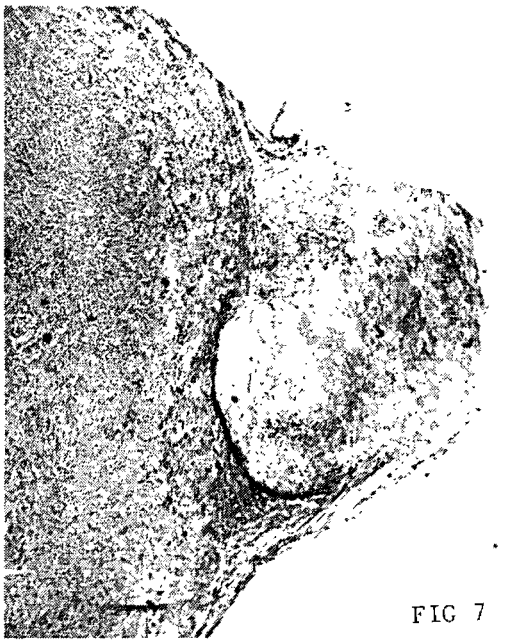

FIG 7

Fibrous ring in the aorta at the level of the top of the sinuses of Valsalva. H E x 30.

*Fig. 8*
Fibroelastic cartilagenous metaplasia of connective tissue in aortic and valvular lesions. Verhoeff's elastic stain x 500.

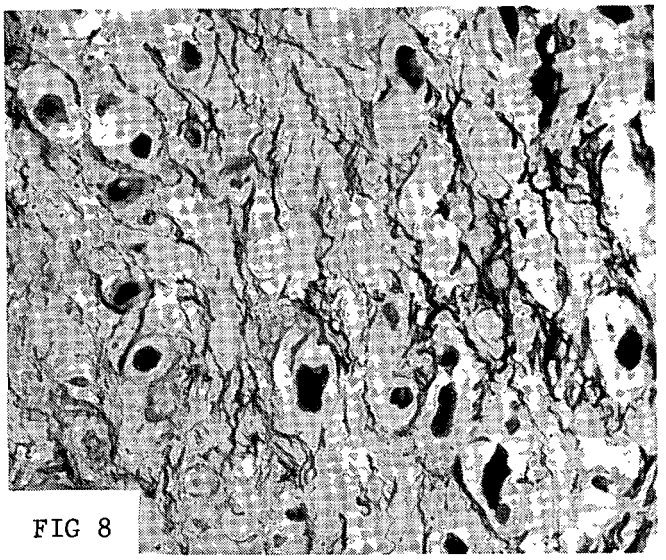

FIG 8

*Fig. 9*
Nodule on aortic semilunar valve. Composed of collagenous connective tissue undergoing metaplasia to fibroelastic cartilage H E x 75.

FIG 9

was held closed for periods of up to 15 seconds to eliminate respiratory interference. On the phonocardiogram the first sound was visible as a separate entity, and was followed 0,03 seconds later by a "diamond shaped" (crescendo - decrescendo) systolic murmur lasting 0,15 seconds. The second sound was visible as a separate entity (Fig. 3). The murmur was also recorded over the carotid arteries.

## RADIOGRAPHY

Radiographs were exposed with the dog in the dorsoventral and lateral positions as described by Ettinger and Suter[4].

On the lateral view a grossly dilated aortic arch, slight elevation of the trachea and slight enlargment of the left ventricle were visible. The cranial waist of the heart shadow was absent.

On the dorsoventral view the cardiac silhouette was elongated by a very prominent and bulging aortic arch.

## PROVISIONAL DIAGNOSIS

On the basis of the typical murmur, the severely dilated aortic arch and the syncopial episodes, a provisional diagnosis of aortic stenosis was made. The ECG was confusing and was ignored at this stage.

## CARDIAC CATHETERIZATION AND ANGIOGRAPHY

To make a final diagnosis, anaesthesia was induced using a short acting barbiturate, the dog was intubated and anaesthesia was maintained using a gaseous mixture of oxygen and Fluothane**.

Following skin preparation, the right femoral artery was exposed surgically. A No. 6 gauge catheter was passed via the artery into the left ventricle. Considerable difficulty was experienced in passing the catheter between the aortic valves, and during this manipulation a test radiograph was exposed after injection of 10ml of Urografin 60***. On the radiograph the catheter was seen to have entered the left coronary artery. This had no apparent ill effect on the dog. When the catheter tip had entered the left ventricle, as ascertained by fluoroscopic examination, 10ml quantities of Urografin were injected using a syringe and maximum manual pressure. Radiographs were exposed as the injection was completed. (Neither pressure injector nor rapid plate changer was available.) Angiograms so obtained (Fig. 4) showed narrowing in the region of the aortic valves and gross post stenotic dilation of the aorta and the left subclavian and brachiocephalic arteries. Systolic pressure measurements were recorded as the catheter was withdrawn. These were 240mm Hg for ventricular systole and 140mm for aortic systole.

The pressure gradient and the radiographic picture confirmed the diagnosis of aortic stenosis.

## PROGNOSIS

The prognosis without surgical interference was poor. Since surgery required extracorporeal circulation and the necessary equipment was not available, correction could not be undertaken. The dog would probably have died of congestive heart failure or ventricular fibrillation[4]. For these reasons the owner requested euthanasia.

"Intraval Sodium" (Thiopentone sodium) Maybaker.
**Halothane, I.C.I.
***Amidotrizoate 76%, Schering.

NECROPSY FINDINGS:

(a) *Gross*

The heart weighed 194g. There was a severe concentric left ventricular hypertrophy, the heart wall measuring 23mm in thickness at the level of the mitral valve and 10mm at the apex. Aortic cusps were thickened, wrinkled and curled (Fig. 5), and little elasticity could be demonstrated. Inside the aorta, at the level of the top of the sinuses of Valsalva, a dense ring of tissue projected into the lumen of the aorta (Fig. 6). The right semilunar valve had a hard nodule on the ventral surface which was 2mm in diameter and there was a similar nodule on the aortic wall in the septal sinus of Valsalva (Fig. 7). The nodule on the right semilunar valve was not related to the lunula. No jet lesions were seen on the endocardium.

(b) *Microscopic*

The projecting ring and nodule in the aorta were confined to the intima and were well demarcated from smooth muscle fibres of the media (Fig. 7). They consisted of collagenous connective tissue rich in a ground substance which was basophilic when stained with haemotoxylin eosin and positive for acid muco-polysaccharide when stained with alcian blue. Distributed throughout the ring and nodule were large cells partially surrounded by clear spaces, giving the appearance of the lacunae of cartilage (Fig. 8). Elastic fibres were demonstrated in association with these large cells by use of Verhoeff's elastic stain(Fig. 8).

The nodules on the semilunar valves were morphologically similar to the aortic lesion but contained less ground substance (Fig 9).

The cusps were diffusely thickened, the dorsal fibrous portions by increased collagen and the ventral spongy portions by myxomatous tissue and collagen. Elastic fibres extending from the endocardium of the ventricle into the semilunar valves were separated and fragmented.

DISCUSSION

Aortic stenosis is a relatively common cardiac defect amongst dogs[4]. It is usually congenital and subvalvular[5]. The Boxer, German Shepherd[4 6] and Newfoundland[4] breeds are predisposed to congenital subaortic stenosis. Valvular and supravalvular lesions have also been described[4]. In this case the lesions were valvular.

The cause of the lesions could not be determined. Microscopic changes were end-stage in nature, consisting of scar tissue undergoing fibrocartilagenous metaplasia. There was none of the inflammatory infiltrate or neocapillary proliferation which would be expected if an infectious agent had been involved. Considering the degree of change present and the youth of the dog, a congenital condition possibly related to improper, or absent, differentiation of mesenchymal tissue making up the valves seemed likely.

Many cases of aortic stenosis are asymptomatic[4]. Where present, symptoms usually include syncope, excessive panting, coughing and pulmonary congestion[4]. This dog had syncope and panted excessively. Syncope in such cases is presumed to be due to lack of left ventricular reserve[1].

The slightly prolonged P wave was considered indicative of mild left atrial dilation. This case was unusual in that the ECG included bizarre QRS complexes which were interpreted as a right bundle branch block pattern. This may have been the result of left ventricular hypertrophy of such severity that the electrical axis swung past -90°resulting in a false right bundle branch block pattern. The slight ST segment changes and the large but variable T wave may have been indicative of a slight degree of myocardial ischaemia at rest.

During syncope, the absence of P waves with a fairly regular and relatively slow heart rate was interpreted as atrial standstill [2]. The radical ST segment changes were presumably due to myocardial ischaemia.

The phonocardiographic findings were typical of aortic stenosis [4]. Translesional pressure gradients recorded in other cases have varied between 30 and 200mm Hg[4]. This case had a gradient of 100mm Hg.

Post stenotic dilation of the aorta in such cases is variable and may be impossible to ascertain on plain radiographs[4] . In such cases measurement of the "A/S ratio" on angiograms can be helpful. The "A/S ratio" is the maximum diameter of the aorta after the stenosis over the diameter of the aorta at the sinuses of Valsalva. In aortic stenosis the A/S ratio is more than I [3]. In this case it was 1,6.

ACKNOWLEDGEMENTS

The authors thank: Dr. G.R. Bolton of the New York State Veterinary College, Ithaca, New York for advice and interpretation of the electrocardiograms;

Dr. I.P.L. Dannheimer and staff of the Cardiology Unit, H.F. Verwoerd Hospital, Pretoria, for technical assistance and encouragement;

Professor I.W. Simpson, Dept. of Pathology, Faculty of Medicine, University of Pretoria, for help in interpreting the microscopic valvular changes;

Dr. C.J. Roos and Staff, Dept. of Surgery, Faculty of Veterinary Science, University of Pretoria for aid in preparation of radiographs and angiograms, and Miss M. Kruger for her technical help.

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Jl S.Afr. vet. Ass. 46(3) 295-297 1975

CASE REPORT GEVALVERSLAG

# ULCERATIVE GLOSSITIS — A FACET OF FELINE PANLEUKOPENIA

MAUREEN K. BAKER*

SUMMARY

The clinical findings and the macroscopic and microscopic lesions in a kitten which died during an outbreak of ulcerative glossitis in a cattery are described. A brief review of the literature is given to support the theory that the virus of feline panleukopenia may well be the aetiological agent in this outbreak.

## OWNER HISTORY

On 15.1.1974 two fortnight old orphan kittens from Cattery A were introduced into Cattery B, which was occupied by a number of adults together with 28 healthy kittens, in order that they might suckle off a queen with a litter of the same age. Approximately 10 days after the two kittens had been introduced, the breeder found that they were lethargic and salivating and on examination "red spots" were found on the dorsal aspect of the tips of the tongues. The two kittens were thereupon returned to Cattery A and subsequently died.

Two weeks later, a similar syndrome appeared in kittens present in Cattery B and within 3 weeks nine kittens had developed tongue lesions. The lesions developed on the tip of the tongue dorsum and were at first of approximately match head size. Subsequently they spread around the sides of the tongue, until finally after approximately 10 days the lingual edges appeared swollen and "waxy-white". This waxiness eventually sloughed off to leave a raw tender area. The lesions then regressed and healed within 21 days of their first appearance. At no time was any nasal or ocular discharge or diarrhoea observed amongst the affected kittens.

Other signs of illness reported were lassitude and salivation. The breeder reported feeling hard "lumps" in the abdomens of the affected kittens and dosed them with cod liver oil and manually extracted the faeces. During the period of illness, a commercial invalid food, Complan* mixed with milk and water was force fed. In addition one or a combination of the following drugs was administered to the affected kittens: lincomycin, chloramphenicol and ampicillin by parenteral injection, ampicillin, sulphadimidine, proteplex**, vitamin C and vitamin B per os and the topical application of a solution of gentian violet to the ulcerative lesions.

Three of the nine affected kittens died; only one of these kittens was available for autopsy.

It is interesting that in Cattery B the condition occurred in two kittens as early as 2 weeks of age, whereas the remainder of the affected kittens were 6-8 weeks old. In some cases whole litters were affected, in others only part of the litter. All queens were apparently healthy throughout the duration of the enzootic.

It is perhaps significant to record that the affected kittens were the progeny of queens whose ages varied from 4 - 6 years and which had been inoculated against feline panleukopenia (FPL) 1-3 years previously. It was the breeder's policy to inoculate all breeding stock annually with an inactivated vaccine until they reached the age of 3 years.

## CLINICAL HISTORY

Three weeks after the commencement of the outbreak, four of the affected kittens were brought to the Department of Medicine, Faculty of Veterinary Science, University of Pretoria.

During examination, one male 9 weeks of age, died. The cadaver was frozen until autopsy 24 hours later, when the anterior two-thirds of the tongue was excised and stored at -20°C for virological examination. The other three kittens were taken home. Before death the salient clinical findings were salivation, lassitude, ulcerative glossitis, dehydration and constipation.

## LABORATORY FINDINGS

Bacterial cultures made from the tongue yielded a rough strain of *Escherichia coli*.

## PATHOLOGICAL FINDINGS

### *GROSS*

The only lesions of significance were ulcerative glossitis particularly of the tip and sides of the anterior two-thirds of the tongue which resembled that described above, generalized atrophy of lymph nodes and oedema and congestion of the lungs. Specimens

* c/o Dept. Infectious Diseases, Fac. Veterinary Science, Univ. of Pretoria Box 12580, Onderstepoort 0110

* Glaxo-Allenburys (S.A.)(Pty.) Ltd.

** Burns (S.A. Cyanamid(Pty.)Ltd.)

from all lesions and major organs were taken and fixed in 10% formalin for histopathological examination

*HISTOPATHOLOGY*

The tongue lesion, examined microscopically, consisted of a focal area of necrosis of the epithelium which in its centre involved cells of all layers and was manifested chiefly by karyorrhexis or karyolysis and cytoplasmic eosinophilia although in some areas cell detail was absent and superficial desquamation had occurred. There was no associated inflammatory reaction but several nuclei of epithelial cells bordering the necrotic area contained single small eosinophilic inclusion bodies.

The lungs showed mild inflammation, chiefly interstitial in location. Infiltrating cells were mainly mononuclear. The pneumonia was accompanied by rather severe oedema.

As it is considered that the freezing and subsequent thawing of the carcase might have influenced possible pathological changes in the other organs examined these are not included in this discussion.

## DISCUSSION

Ulcerative glossitis in cats has been known for some time. The condition was first described by Smythe [11] in 1934. Subsequently Kirk [7], Weipers [12], Joshua [6] and Wilkinson [13] mentioned the condition and the disease. All five of the above authors suggested a relationship between this condition and FPL, an assumption which was borne out by the fact that cats which had recovered from ulcerative glossitis were apparently immune to FPL. According to Joshua (personal communication) the condition is most frequently seen in cats between the age of 18 months and 3 years.

Both Joshua [6] and Wilkinson [13] reported that the disease is rarely fatal. In the outbreak under discussion only three of the nine kittens died. The dehydration and constipation of the autopsied kitten can be attributed to the tongue lesions which made lapping painful.

Rohovsky and Fowler [10] in a study of FPL in germ-free cats reported that none of the inoculated cats died and that enteritis did not occur. Thus it would appear that enteritis in FPL is not due primarily to the virus but due to bacteria present in the gastro-intestinal tract. The variety of antibiotics used by the breeder would certainly have depressed, if not modified, the intestinal flora in the affected kittens. The absence of lesions in the small intestine in this case is in marked contradistinction to classical FPL but nevertheless does not preclude a diagnosis of FPL.

In a survey [1], it was reported that the characteristic sternal recumbency or "praying position" and the hanging posture of the head over the water bowl was considered to be a significant diagnostic sign of FPL. It was further reported that mouth and tongue ulcers sometimes accompanied FPL. Carpenter [3] reported that a variety of lesions, including necrotic gingivitis and ulcerative glossitis, may occur in cats affected with FPL.

Langheinrich and Nielsen [8] commenting on the significance of intranuclear inclusion bodies in FPL considered their presence to be diagnostic, especially when correlated with the other histological lesions, but stated that they may be absent. The associated histopathological changes which they described included reticuloendothelial hyperplasia, lymphocytic depletion, loss of follicular architecture, haemorrhage, congestion, reticuloendothelial hypoplasia and plasma cell infiltration in the spleen. There is in addition a severe depletion of neutrophils, while the occurrence of erythrophagocytosis in lymph nodes and the spleen in FPL is common knowledge.

Glossitis has been reported in a number of other feline viral diseases. Wilkinson [13] reported ulceration of the tongue in feline viral rhinotracheitis (FVR). No lesions of the upper respiratory system were noticed in the cat reported on here. Hoover and Griesemer [4] working with germ-free cats reported that severe upper respiratory symptoms were a constant finding in FVR-infected cats.

Hoover and Kahn [5] investigated the lesions produced by feline picornaviruses of high and low virulence in pathogen-free cats and reported that ulcerative glossitis occurred in both cases. Furthermore, the highly-virulent virus always produced pneumonia, whereas the virus of low virulence produced mainly a lingual ulceration. They did not mention any pathological changes in the liver or spleen.

O'Reilly, Paterson and Harris [9] in an experiment to determine the persistence of maternal antibody to FPL in kittens found that the queens' antibody titres declined in the absence of exposure to natural infection. Furthermore, they found that six out of 10 kittens born to queens with an antibody titre of 1:32 had FPL antibodies 4 weeks after birth, but that by 6 weeks of age only three out of 16 kittens had a detectable antibody titre. After 11 weeks no antibodies were detectable in any of the kittens. In their study the antibody titre levels in the kittens varied from 1:32 to less than 1:8, thus only 28% of kittens from queens with low antibody titres had detectable antibody levels at 6 to 7 weeks of age.

No reference can be found concerning the variation in antibody titres within the same litter of kittens; however, in a study undertaken on piglets, Nordbring and Olsson (cited by Brambell [2], 1970) showed that at 6 weeks of age titres from colostral antibody to a paratyphoid vaccine in a litter of piglets varied between 1:160 to 1:320. Since both these species are polytocous, an analogous situation may well exist in cats.

The demonstration of eosinophilic intranuclear inclusion bodies in the lingual epithelium supports the theory that FPL virus, or a strain thereof, may be the aetiological agent in this outbreak of ulcerative glossitis.

In any attempt to explain the pathogenesis of ulcerative glossitis and its relationship to classical FPL there would appear to be an interesting interrelationship between the waning antibody levels of the affected kittens, the predilection of the virus for rapidly multiplying cells and the particular location of the lesions, which in this situation appears to be confined to the tongue – an organ whose epithelial surface is subject to considerable cellular replacement and possibly a mild degree of trauma in the suckling kitten.

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# BOOK REVIEW

# RESENSIE

## ECOLOGY AND CONTROL OF RODENTS OF PUBLIC HEALTH IMPORTANCE

TECHNICAL REPORT SERIES NO. 553 W.H.O. 1974

pp. 42, Annex 1, Tables 1, Price SW fr. 5. -

This Report of a WHO Scientific Group which met in 1973 deals in a brief concise and systematic way with the importance of rodents in public health as vectors of diseases transmissible to man, the ecology of these rodents, control and management of rodents, and recommendations. It includes a chapter on Information and Training of the public and rodent control personnel, and an Annexure with a table providing detailed information on the relevant rodent species and the associated human disease. A list of 78 references is provided for persons who require further detailed information.

The report is a valuable document for all who are interested in Zoonoses, Environmental Health and Sanitation

L.W. v.d. H.

## Chimpanzee-Associated Hepatitis

Between May 22 and May 28, 1974, 5 persons in Cumberland County, Pennsylvania, developed jaundice, and diagnoses of hepatitis were made. Serum specimens obtained from these individuals and tested for hepatitis B surface antigen (HBsAg) were negative.

None of the patients had been exposed to known cases of hepatitis, raw shell fish or contaminated food or water. However, all 5 patients had had contact with a young, newly imported chimpanzee. The 12-month-old chimpanzee had arrived at a privately owned zoo on April 10, 1974, thin and highly nervous with dry, scaly skin. Also, she had a poor appetite and persistent diarrhea.

On May 1 the chimp was treated by a local veterinarian and cared for by the assistant of another veterinarian in her home. Subsequently, over a 7-day period in May, the chimpanzee owner (age 53) and his wife (55), a part-time employee (17), the veterianarian's assistant (20) and her boyfriend (24), all of whom had frequent contact with the chimpanzee, developed acute HBsAg-negative hepatitis.

Fifty-two contacts of the 5 patients received immune serum globulin (ISG). No additional cases of hepatitis occured.

A blood specimen obtained from the implicated chimpanzee revealed an SGOT of 85 IU (chimpanzee normal 0-15 IU) and a bilirubin of 2.0 mg% (chimpanzee normal 0.1-0.5%). A cage-mate of the implicated chimpanzee appeared healthy, but blood tests revealed a normal bilirubin with an SGOT of 81 IU.

Since the first reports of nonhuman primate-associated hepatitis in the early 1960s, over 200 cases in humans have been reported, and the frequency of such reports seems to be increasing. In 1974, 8 outbreaks in 7 states were reported to CDC. The disease is usually mild, of brief duration, and clinically indistinguishable from hepatitis A. Various nonhuman primates have been associated with cases of hepatitis in humans, but the most frequently implicated have been chimpanzees that were newly imported and appeared well or had nonspecific clinical illness.

Persons who must work with newly imported chimps are advised to maintain scrupulous personal hygiene, and since immune serum globulin seems to protect animal handlers against clinical hepatitis, they should receive ISG routinely.

Source: Center for Disease Control (U.S. Dept. of Health, Education and Welfare): Morbidity and Mortality Weekly Report 24(12):22 March 1975

## TOXICOLOGICAL EVALUATION OF CERTAIN FOOD ADDITIVES WITH A REVIEW OF GENERAL PRINCIPLES AND OF SPECIFICATIONS.

WORLD HEALTH ORGANIZATION TECHNICAL REPORT SERIES NO. 539, GENEVA, 1974.

pp. 40. Tabs. 1 Publ. Price Sw. fr. 5-.

This is an updated report by the joint FAO/WHO expert committee on food additives.

The general principles of testing these relatively harmless but essential substances and the necessity therefore, are emphasized. The approach to the problem in general and specifically to substances with an acute $LD_{50}$ of over 5g/kg on the acute toxicity basis and at 10% and over in the diet on sub-acute toxicity studies is essentially practical.

A safety factor of 100 (with specific reservations) is generally advocated due to the problem of extrapolation of laboratory animal data to man. and is used to give the accepted daily intake (ADI) for man of just over 100 compounds in general use. Certain previously used compounds have been delted from the list and other modifications have been effected as a result of newly acquired toxicity data. It is interesting to note i.a. that monosodium glutamate (and the other glumatic acid salts) are regarded as relatively safe at ADI of 120 mg/kg.

It should be a very valuable guideline for health authorities in this field. T.W.N.

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## ANAPLASMOSIS

A veterinary scientist of the Mississippi Agricultural and Forestry Experiment Station (MAFES) is using neutron bombardment of blood parasites in seeking a cure for anaplasmosis. The experimental work is being carried out by John N. Love, assistant bacteriologist in the MAFES Veterinary Science Department and R.M. Rubin, of Mississippi State University's Department of Nuclear Engineering.

Anaplasmosis is a disease in which a minute parasite body invades the red blood cells of a ruminant animal. Horseflies and ticks are the primary vectors responsible for spreading the desease from infected to uninfected animals. Broad spectrum tetracycline antibiotics are currently the best available treatment. Vaccines so far developed have been partly successful but do not eliminate the development of the carrier stage of anaplasmosis. Mr Love is attempting to develop a vaccine that will eliminate this stage of the disease.

Other researchers had used gamma rays to kill the parasites in a sample of infected blood, but no immunity was produced. However, since neutrons are more effective in causing biological damage than gamma rays, a lower dose of neutrons might modify the parasites in a blood sample, making them less virulent and creating an effective vaccine.

Research on anaplasmosis has been made easier by results determined during an earlier phase of investigation:

1) Calves below one year of age do not usually develop acute cases of anaplasmosis as do older cows, but after removal of the spleen, the calf will develop a virulent case of anaplasmosis after inoculation with infected blood and can be readily used in research.

2) By using liquid nitrogen to freeze infected blood, the blood could be preserved indefinitely. The anaplasma parasites were still active upon thawing of the blood. This means that once a supply of infected blood is frozen, it is not necessary to keep on hand an animal with acute anaplasmosis.

Older animals are much more susceptible so that clinical manifestation of the disease in animals under 18 months of age is rather rare. Herd bulls and mature cows are much more likely to get this disease and, if not treated, it is usually fatal among cattle three years old and over.

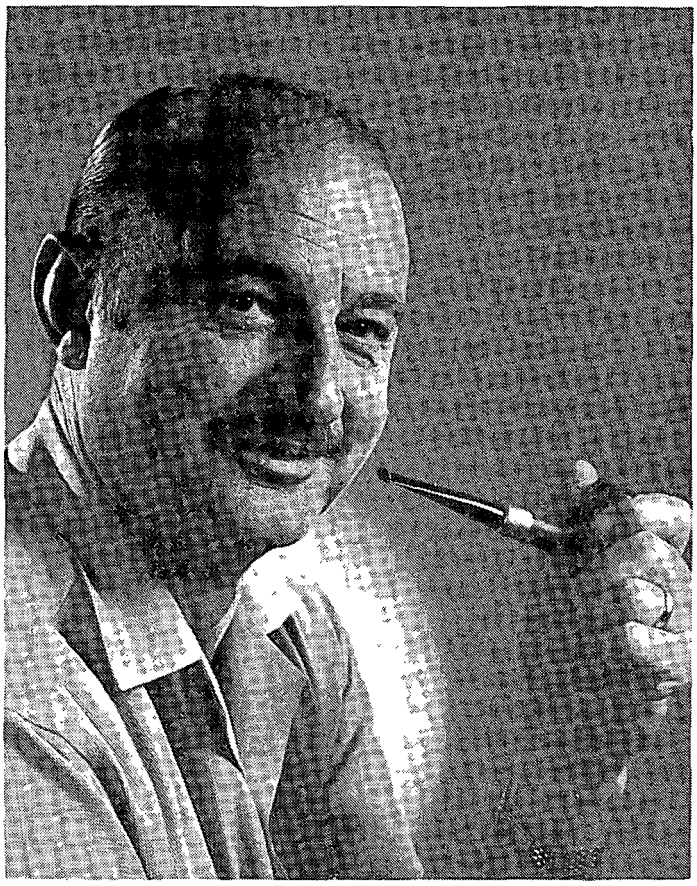
Since only a small droplet of blood from an infected animal is needed to infect a healthy animal, man is probably the most effective spreader of anaplasmosis. This is done through the careless use of instruments used in de-horning, tattooing, bleeding or vaccinating.

The irradiation of parasitic bodies by neutron bombardment has not yet produced the ultimate vaccine for anaplasmosis. However, it has modified the parasitic bodies so that, when a calf is injected with the vaccine, it takes three weeks longer than with the un-modified blood for the calf to begin showing the signs of anaplasmosis - anemia, increased body temperature, weakness, depression, dehydration, loss of appetite, laboured breathing and irrational behaviour. MAFES researchers feel the concept of irradiation has many possibilities and could ultimately lead to eradication of the disease.

*MAFES Research Highlights*; (April 1975); Agricultural Research Station, Mississippi State University, State College, Miss. 39762.

## IN MEMORIAM

JAKOB JOHANNES OBERHOLSTER 2.1.1926 - 16.1.1975.



Op 16 Januarie 1975 het ons nog 'n vriend en kollega in die fleur van sy lewe as gevolg van 'n motorongeluk verloor. Brollie, soos hy by baie van ons bekend was, was met sy vrou en dogter op pad na Potchefstroom toe die motor op die nat pad gegly het.

Jakob Oberholster is op 2 Januarie 1926 in die distrik Outjo, SWA gebore. Reeds vanaf sy vroegste jare was hy 'n briljante skolier en matrikuleer te Windhoek in die eerste klas met drie onderskeidings. Die B.V.Sc.-graad behaal hy aan die Universiteit van Pretoria in 1949 ook met lof. Daarna begin hy sy loopbaan as Staatsveearts in Suidwes-Afrika maar besluit gou dat hy vir 'n plattelandse praktyk bestem is. In 1951 vestig hy hom op Bethlehem waar hy dan ook tot sy dood gepraktiseer het.

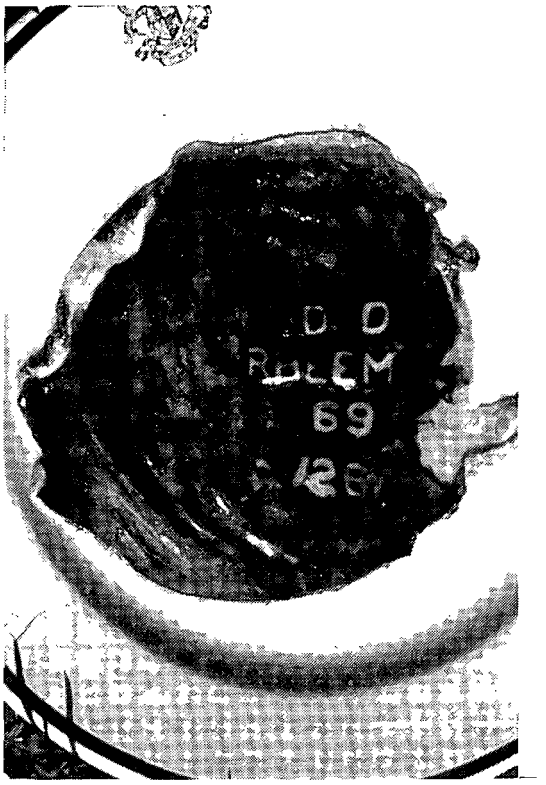
Brollie was die verpersoonliking van die plattelandse praktisyn in Suid-Afrika. Die verhouding tussen hom en die boeregemeenskap was baie meer op 'n vrienskaplike grondslag as die normale besigheidsverhouding tussen veearts en kliënt. Om dié rede was hy 'n baie gewilde praktisyn.

In die bietjie vrye tyd wat hy as alleenpraktiserende veearts tot sy beskikking gehad het, het hy 'n pistoolklub op Bethlehem gestig wat nou na sy dood na hom vernoem is. Gelukkig was die geleentheid hom nog gegun om die hoogste trap in hierdie sportsoort te bereik toe hy in 1974 as Springbokpistoolskut gekies is om Suid-Afrika op die wêreldkampioenskapstoernooi is Switserland te verteenwoordig - die derde veearts wat daarin geslaag het om die groen-en-goud te dra.

Namens die professie wil ons aan sy vrou, Rikie, sy dogter, Benita, en sy seuns, Dewald en Derek, ons innige meegevoel betuig met hulle groot verlies.

## FEATURE PAGE

## TREFFERBLAD




### THE WRITING ON THE (RUMEN) WALL.

A remarkable case of lettering on the rumen wall was reported to this Regional Veterinary Laboratory on the 9th of May 1974.

The subject was a two-tooth cross-bred (Dorper x Merino) wether which was born and bred on a farm in the Middelburg (Cape) district and kept on natural grazing until the day before slaughter. The owner, who also owns a local hotel, was not present at the time of slaughter but only informed later. At the time of his observations the rumen was already scalded for use as tripe. The part in question was removed and brought to the Laboratory.

The portion of the rumen presented showed the heavy pigmentation often found in such cross-bred sheep. In this pigmented area the following figures and letters were distictnctly visible from the peritoneal surface: DD, RHEEM, 69, 1261 (see photograph). Only the H in "Rheem", which was poorly defined, could be seen on inspection of the epithelial surface.

As was confirmed by making a horisontal section through one of the D's, these figures and letters were formed by compelte inhibition of the normal pigment.

The only feasible explanation that can be offered is that this animal swallowed at least part of a plastic container on which these letters and figures were printed. This plastic material probably adhered very closely to, and with the printed side facing the epithelial surface of the ruminal wall. It is postulated that a particular substance in the printing material was absorbed by the ruminal wall and inhibited normal pigment formation or otherwise caused chemical reduction of the colour of the pigment.

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### DIE SKRIF AAN DIE MUUR (RUMENWAND)

Hierdie merkwaardige geval van letterwerk op die wand van die rumen is op 9 Mei 1974 by hierdie Streekslaboratorium aangemeld. Die onderwerp was 'n tweetand Dorper-Merino hamel wat op 'n plaas in die distrik Middelburg, Kaap, gebore en op natuurlike weiding aangehou is tot die dag voor slagting. Die eienaar, wat 'n plaaslike hotel besit, was nie tydens slagting aanwesig nie en sy aandag is eers later op die saak gevestig d.w.s. nadat die pens vir gebruik as voedsel voorberei is. Hy het die betrokke deel verwyder en na die Laboratorium gebring.

Die aangebode deel van die rumen-wand was swaar gepigmenteer soos tewens dikwels die geval by bogenoemde kruisings is. In die gepigmenteerde gedeelte kon die volgende syfers en letters duidelik vanaf die peritoneale aansig gesien word: D D, RHEEM, 69, 1261, (Sien foto). Slegs die H in "Rheem", wat onduidelik omskrewe was, kon vanaf die epiteelaansig gesien word.

Deur 'n horisontale snit deur een van die D- letters te maak kon vasgestel word dat die syfers en letters die gevolg was van algehele gebrek aan pigment.

Die enigste aanvaarbare verklaring vir die verskynsel is dat die dier minstens 'n deel van 'n plastiese houer waarop die genoemde lettermerk gedruk was, ingesluk het. Die materiaal het waarskynlik met die gedrukte vlak teen die epiteel van die rumen-wand vasgekleef. Dit word aangevoer dat 'n sekere bestanddeel van die drukstof deur die rumen-wand opgeneem is en die normale vorming van pigment verhinder het, of andersins verantwoordelik was vir chemiese reduksie van die pigment kleur.

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|---|---|---|---|
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| NODULAR WORM | X | X | A |
| HOOK WORM | X | X | A |
| LIVER FLUKE (ADULT) | | | HIGHLY EFFECTIV |
| GIANT LIVER FLUKE (ADULT) | | | HIGHLY EFFECTIV |



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