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# JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

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**CONGRESS PAPER** **REFERAAT**

# ON THE AETIOLOGY OF JAAGSIEKTE*

D.W. VERWOERD AND E-M. DE VILLIERS

ABSTRACT: Verwoerd D.W.; de Villiers E.-M. **On the aetiology of jaagsiekte.** *Journal of the South African Veterinary Association* (1980) **51** No. 2 71-74 (En) Veterinary Research Institute, 0110 Onderstepoort, Rep. of South Africa.

A summary is given of the results obtained in experimental transmission of jaagsiekte by means of transplantation of cell cultures. Evidence is also presented of transformation as the mechanism of oncogenesis and the possibility of a viral aetiology is discussed briefly.

## INTRODUCTION

The infectious nature of jaagsiekte or ovine pulmonary adenomatosis has been known for at least half a century[11 14]. Nevertheless, none of the many workers in various countries has been able to establish unequivocally even the type of agent responsible for this disease. The demonstration of transmission with filtrates of lung exudates and tissue extracts suggested the involvement of a virus and a number of putative viral agents have indeed been isolated.

All the early work had one factor in common namely the use of tumorous lung tissue as starting point. In order to avoid the complications introduced by the presence of secondary agents in the diseased lung we took a different approach when starting to investigate the disease some years ago. We decided to establish cultures of the tumour cells first and search for the jaagsiekte agent only when we were certain that the cultures were free from contaminating organisms. It turned out to be much more difficult than we expected to grow the epithelial tumour cells but eventually we developed a reasonably efficient technique by means of which eleven cell lines have been established to date[2]. These cell cultures were tested for oncogenicity in newborn lambs as well as in athymic nude mice. All except one produced tumours in either of these hosts, as shown in Table 1[12].

In order to determine whether the tumours produced in lambs consisted of transplanted cells or of the recipient's own cells transformed by an infectious agent introduced with the cells injected, we planned an experiment using the sex chromosomes as markers. Male cells would be injected into female animals and vice versa and the sex of the resultant tumour determined. On karyotyping our 15,4 cell line, which was established from a ewe and used for most of our experiments, it turned out to be male in character. This proved to us that transplantation can take place, even under natural conditions, suggesting that the known infectivity of the disease can, at least in part, be explained by the transmission of live tumour cells from animal to animal. This was supported by the demonstration of large numbers of live tumour cells in the sometimes copious lung exudate which can probably be disseminated easily in the form of an aerosol by animals coughing or sneezing.

*Paper delivered at the South African National and International Veterinary Congress, 1979 held in Johannesburg. Parts of the results presented here have been published elsewhere.

Table 1: EPITHELIAL CELL LINES ESTABLISHED FROM ADENOMATOUS OVINE LUNG TISSUE

| Cell line (sex) | Sex of donor | Cells inoculated | Tumours produced in Lambs | Tumours produced in Nude mice |
|---|---|---|---|---|
| 6,11 (male) | Male | Natural case | NT[1] | NT |
| 15,4 (male) | Female | Natural case | 18/29 | 14/17 |
| 21,3 (female) | Female | Natural case | 6/10 | 0/10 |
| 30,2 (female) | Female | Natural case | 0/2 | 0/10 |
| 29,3 (male) | Male | 15,4 (male) | 1/2 | NT |
| 45,3 (male) | Male | 15,4 (male) | 1/2 | 0/2 |
| 48,3 (male) | Male | 15,4 (male) | 0/2 | 1/10 |
| 58,2 (female) | Female | 15,4 (male) | 2/6 | 0/9 |
| 59,1 (male) | Male | 15,4 (male) | 1/1 | 6/10 |
| 71,1 (male) | Male | 21,3 (female) | NT | 1/6 |
| 69,2 (female) | Female | 15,4 (male) Cell homogenate | NT | 0/10 |

[1] NT – not tested

Further transplantation studies with the other cell lines yielded more interesting results (Table 1). In 8 out of 10 cases the sex of the cell line was identical to that of the donor in field cases and of both donor and injected cells in experimental cases. This is consistent with transplantation as the mechanism of transmission although transformation cannot be excluded.

The female 58,2 cell line, however, originated from the transplantation of male cells into a female animal and vice versa, the male 71,1 line from inoculation of female cells into a male animal. The only possible explanation for this phenomenon is the transformation of the recipient's cells by the transfer of genetic information, viral or otherwise, present in the transplanted cells. Transformation as an alternative mechanism for the oncogenesis of jaagsiekte was confirmed by the transmission of the disease with cell homogenates. Unequivocal proof that these results were not due to the presence of any residual viable cells was provided by the cell line 69,2, derived from a female animal injected with a homogenate of male cells, which was clearly female in its karyotype. All the tumour cell lines were aneuploid, without any characteristic deviation in chromosome number or morphology.

## TRANSPLANTATION AS AETIOLOGICAL FACTOR

The tumorigenicity data given in Table 1 suggest an overall efficiency of transplantation in lambs of about



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50 %. When the figures in Table 1 are analyzed according to the sex of the recipient lambs, however, the transplantation efficiency of male cells seem to be significantly higher in the homologous, compared to the heterologous sex (Table 2). The effect is even more pronounced when the growth rate of the tumours are taken into account (Table 3). In male recipients transplantation of male cells produced fast-growing tumours with extensive lesions often involving a third of the lungs or more after 6 to 12 months. In contrast, the same cells produced mainly early type lesions which could only be recognised histologically after 12 months in female recipients.

In the case of female cells the number of experimental animals involved were smaller and the results consequently more difficult to interpret. There does not seem to be a significant difference, however, between either the efficiencies of transplantation (Table 2) or the growth rates of the tumours (Table 3) between recipients of the two sexes, even though the tumorigenicity of the female cells seems to be lower than that of male cells.

In the experiment summarized in Table 4 an attempt was made to improve the efficiency of transplantation of 15,4 cells by suppressing the cellular immunity usually responsible for the rejection of tumour transplants. Anti-thymocyte serum (ATS) and anti-macrophage serum (AMS) were used in various combinations as shown. The efficiency of these reagents in lambs has been demonstrated[1]. In addition, silica particles of defined size distribution were administered intratracheally in an attempt to inactivate alveolar macrophages.

All the male lambs not receiving immunosuppressive treatment developed extensive lesions whereas the small number of equivalent females that were positive had small lesions, confirming the trend shown in Table 3. Treatment with ATS could not further facilitate the transplantation in male recipients but in the case of females it did seem to enhance both the number of positives and the tumour growth rate. Treatment with AMS blocked transplantation in both sexes. Silica dust did not have any significant effect as far as can be judged from the small numbers of animals involved.

The enhancing effect of ATS suggests that the lower efficiency of transplantation of male cells in females is due to an immunological rejection mechanism. The observed phenomenon could well be due to the presence of a surface antigen determined by the Y-chromosome in male cells. Such cells would be recognised as foreign and rejected in female animals but not in males. Female cells, on the other hand, would not possess this antigen and should be accepted equally well by both sexes. Such an antigen, termed the H-Y antigen, has in fact been demonstrated in mice and various other animals[10]. In most cases, however, it is a rather "weak" antigen playing a minor role in transplantation compared to the dominant histo-compatibility antigens. Little is known about either the H-Y antigen or the transplantation antigens of sheep, therefore their relative importance in the transplantation studies described cannot be evaluated.

Table 2: THE EFFICIENCY OF TRANSMISSION OF JAAGSIEKTE TO NEWBORN LAMBS BY MEANS OF INTRATRACHEAL INJECTIONS OF TUMOUR CELLS AND CELL HOMOGENATES AS A FUNCTION OF THE SEX OF BOTH THE CELL LINE AND THE RECIPIENT

| | Male recipients | | Female recipients | |
|---|---|---|---|---|
| Cell lines | Transmissions | Efficiency | Transmissions | Efficiency |
| Male: 15,4; 29,3; 45,3; 59,1; 48,3 | 13/17 | 76 % | 8/19 | 42 % |
| Female: 21,3; 58,2; 30,2 | 3/8 | 37 % | 5/10 | 50 % |
| Cell homogenate (15,4) | 3/5 | 60 % | 1/3 | 33 % |

Table 3: GROWTH RATES OF TUMOURS PRODUCED IN MALE AND FEMALE LAMBS BY INTRATRACHEAL INJECTION OF MALE AND FEMALE CELLS

| | Male cells | | Female cells | |
|---|---|---|---|---|
| Growth rate of tumour | Male lambs | Female lambs | Male lambs | Female lambs |
| 4+: Extensive lesions within 6 months | 2 | 0 | 2 | 0 |
| 3+: Extensive lesions after 12 months | 9 | 2 | 0 | 1 |
| 2+: Small lesions after 12 months | 2 | 3 | 1 | 2 |
| 1+: Early lesions after 12 months | 0 | 4 | 0 | 0 |

Table 4: INFLUENCE OF IMMUNOSUPPRESSION ON TRANSMISSION OF JAAGSIEKTE TO NEW-BORN LAMBS BY TRANSPLANTATION OF 15,4 CELLS

| | Males | | Females | |
|---|---|---|---|---|
| Immunosuppression | Transmissions | Average growth rate | Transmissions | Average growth rate |
| None | 5/5 | +++ | 3/9 | + |
| ATS (D−1, +1, +2, +5, +7) | 1/3 | +++ | 1/2 | ++ |
| AMS (D−1, +1, +3, +5, +7) | 0/2 | − | 0/2 | − |
| ATS+AMS (D−1, +1, +2, +5, +7) | 0/3 | − | 0/2 | − |
| ATS (D−3 to D+21) | 2/2 | ++++ | 2/3 | ++ |
| ATS+AMS (D−3 to D+21) | 2/2 | ++++ | 1/2 | ++ |
| ATS+SILICA | 2/2 | ++ | NT | − |
| SILICA | 2/2 | +++ | 0/1 | − |

D = days on which immonusuppressive treatment was given

It should be noted that the injection of tumour cells into the lungs, where they presumably attach to and proliferate on the surface of the alveolar epithelium, cannot be regarded as a normal transplantation process, subjected to the usual rejection reactions. This is borne out by the relatively high percentage of tumour development found in animals that were completely random-bred and also genetically unrelated to the injected cells. The relative absence of normal immunological rejection reactions in the lung alveoli is also illustrated by the fact that a number of lambs which were injected simultaneously intratracheally and subcutaneously with 15,4 cells developed jaagsiekte lesions in the lung but never any subcutaneous tumours.

With the exception of ATS treatment in female lambs receiving male cells, attempts to increase the transplantation efficiency by immunosuppression failed. Treatment with AMS alone or in combination with a short course af ATS seemed to block the transplantation of 15,4 cells in both male and female recipients completely. More extensive treatment with ATS apparently neutralized this effect. These results suggest a role for the thymocytes in a sex-dependent rejection of tumour cells and an enhancing role for the alveolar macrophages in susceptible recipients.

The fact that jaagsiekte was found to be transmissible by means of cell transplantation obviously led us to consider the possibility of immunizing sheep against the disease by means of a cellular vaccine even though we had no idea of the relative importance of the two mechanisms of transmission under field conditions. Preliminary studies on a small number of animals at Onderstepoort, using live 15,4 cells injected subcutaneously as vaccine, looked promising. We therefore proceeded to a large scale experiment on a farm in the Cathcart area with an exceptionally high incidence of jaagsiekte. For safety reasons glutaraldehyde fixed cells were used as a single subcutaneous injection in lambs within a week after birth. The results shown in Table 5 indicate a complete failure to protect the animals. Many possible reasons for the failure can be advanced and many avenues for possible improvement explored but the amount of work involved precluded further work in this direction until we know more about the aetiology of the disease under field conditions.

Table 5: PROTECTION AFFORDED BY A CELLULAR VACCINE

| Treatment | Number of lambs | Cases after 2 years | % incidence |
|---|---|---|---|
| Vaccinated | 266 | 52 | 19,5 |
| Controls | 167 | 38 | 22,7 |

## TRANSFORMATION AND A POSSIBLE VIRAL AETIOLOGY FOR JAAGSIEKTE

Previous mention has been made of evidence for a second mechanism by which jaagsiekte can be transmitted i.e. the transformation of the host's own cells. Early filtration experiments indicated that an infectious agent with virus-like properties is present in lung fluid and in tumour extracts. Many attempts have since been made in various laboratories to demonstrate or isolate a putative virus with some degree of success. I would like to discuss briefly 4 candidate viruses and the experimental results obtained in attempts to prove or disprove their involvement in the aetiology of jaagsiekte.

A herpesvirus was first demonstrated in Scotland in macrophages obtained from jaagsiekte lungs[6]. Limited multiplication and transmission to normal macrophages were also obtained. A morphologically similar virus was later isolated by Malmquist and co-workers in the USA from material obtained in Kenya and was shown to replicate in normal sheep lung fibroblasts[7]. Other reports of herpes-like virus particles in adenomatous sheep lungs came from Chechoslovakia and Bulgaria.

In our own laboratory we have isolated *Herpesvirus ovis* on 4 occasions in as many years from different animals suffering from jaagsiekte[3]. Two isolates were found in fibroblastoid cell cultures established from jaagsiekte lungs, one was rescued after a transient spontaneous induction in an epithelial tumour cell culture and one was isolated from the lung exudate. Many attempts to isolate the virus from normal lungs using various techniques to activate latent viruses failed, suggesting possible involvement in the aetiology of the disease. Serological studies on three of the isolates demonstrated their identity and also that they are either identical or closely related to the virus isolated in Scotland[13]. All attempts by both groups to transmit the disease experimentally by inoculation of lambs with the virus by various routes failed. A survey of sheep sera obtained from various parts of Southern Africa revealed that a high percentage (about 70 %) of all sheep have antibodies against the virus, which seems to be ubiquitous in its distribution. This phenomenon is reminiscent of the position of the Epstein-Barr herpesvirus in the human population, where up to 90 % of people in most countries possess antibodies to the virus although it is implicated in the aetiology of Burkitt's lymphoma and nasopharyngeal carcinoma in only a few tropical areas[5]. However, in contrast to its human counterpart, we did not find higher antibody titres in animals suffering from jaagsiekte. We also tested a number of sera obtained from Iceland, a country known to be free from jaagsiekte. If antibodies against *Herpesvirus ovis* were found in these sera it would have ruled out a role for the virus in this disease but this was not the case. Serological studies therefore neither proved nor disproved involvement of the virus.

Further investigations included attempts to demonstrate the presence of the viral genome in the tumour cells. No viral antigens could be demonstrated and molecular hybridization experiments also yielded negative results[4]. Consequently, our interpretation is that *Herpesvirus ovis* is probably just another latent herpesvirus present as a passenger in jaagsiekte lungs, possibly playing a role as cofactor for some other still unknown agent.

The second candidate is a retrovirus, i.e. a member of the group of RNA tumour viruses mainly associated with leukemias and sarcomas in various animals and characterized by the possession of RNA-dependant DNA polymerase (RDP), commonly known as reverse transcriptase. The first indication for the involvement of a retrovirus was the electron-microscopic demonstration of typical type C particles in jaagsiekte lung lesions by workers in Israel. The same group later demonstrated the presence of RDP activity in extracts of these lesions[8]. Morphologically typical retroviruses were also demonstrated in the cell cultures made in the USA from Kenyan material[7] but there is a strong possibility

that this virus could be identical to the agent causing progressive pneumonia or maedi in sheep, a disease probably present in East Africa. Virus particles and RDP-activity was also recently demonstrated in Scotland in the lung fluid collected from sheep suffering from jaagsiekte[9]. None of these groups could thus far isolate or cultivate their virus and consequently transmission studies have not been reported.

We were able to confirm the presence of reverse transcriptase in the lung exudate of sheep with advanced jaagsiekte lesions but could not demonstrate the enzyme in the infective lung extracts. Particles resembling retroviruses were also found in both lung lesions and in lung exudate by means of electron-microscopy. All attempts to rescue the virus by various means in a variety of cell cultures have been unsuccessful to date. Nevertheless, a retrovirus is probably the leading contender as causal agent for jaagsiekte at present.

The inclusion of a papilloma virus as possible candidate is rather speculative and mainly based on the fact that the histological lesion of jaagsiekte, i.e. a papillomatous proliferation of the alveolar epithelium is very similar to that associated with most papilloma viruses. In addition, bovine papilloma virus (BPV) has been shown to cause various tumours other than warts in foreign species. Attempts were therefore made to produce jaagsiekte by intratracheal injections of bovine papilloma virus but to no avail. Molecular hybridization studies aimed at the demonstration of BPV-related genetic information in the DNA of tumour cells were also unsuccessful.

Finally, there is a possibility that a subviral infectious entity could be involved in the etiology of jaagsiekte. This is mainly based on experiments involving the progressive fractionation of tumour cells combined with testing for oncogenicity in new-born lambs. In this way we have derived a cytoplasmic fraction which is highly oncogenic but does not contain any demonstrable virus and from which we have been unable to rescue any virus. This fraction has many of the characteristics described for the so-called "scrapie-agent" such as relative stability, association with cellular membranes and an extended lag phase. It could consist of a defective viral genome (commonly found in the retrovirus group) but could also represent a non-viral nucleic acid molecule such as that presently believed to constitute the scrapie-agent or similar to the viroids involved in various plant diseases. We have, however, not yet been able to obtain any experimental evidence in support of such a hypothesis.

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**CONGRESS PAPER** **REFERAAT**

# SKEMA VIR DIE BEHEER EN UITEINDELIKE UITROEIING VAN BEESBRUCELLOSE

P.P. BOSMAN*

ABSTRACT: Bosman P.P. **Scheme for the control and eventual eradication of bovine brucellosis.** *Journal of the South African Veterinary Association* (1980) **51** No 2 75-79 Division of Veterinary Services, Department of Agriculture, P/Bag X138, 0001 Pretoria, Rep. of South Africa.

The national incidence of brucellosis – estimated at 6 % – is incompatible with optimal production of milk and beef. This fact and the demands of international trade necessitated the launching of a national scheme for the eradication of brucellosis. Such a scheme was. in fact, announced during 1968 but little progress was made until 1976.

Since then. adaptations to the department's approach to brucellosis control include compulsory branding (a conspicuous "C" on the right side of the neck) of all reactors, the abolition of charges for laboratory tests and (voluntary) accreditation of CA free herds. Considerable progress has also been made with the standardisation of serological tests employed for the diagnosis of brucellosis – viz the Rose-Bengal plate test for individual and the Milk Ring test for herd screening purposes and the microtitre Complement Fixation test as the definitive test.

During April 1979 the launching of the official scheme which was submitted to the Department in January of the same year, was approved in principle and funds, specifically earmarked for the scheme, have since been allocated for the fiscal year starting on the first of April.

The scheme will be based on

1. compulsory S19 vaccination of heifer calves between the ages of three and seven months;
2. selective adult vaccinations (whole herd), possibly with smaller doses of S19 vaccine;
3. identification and branding of reactors. For the time being, slaughter of these animals will be on a voluntary basis with no compensation being paid. This aspect is subject to review, depending on progress;
4. accreditation of herds in order to provide a source of replacement stock;
5. consideration of the institution of an incentive scheme to stimulate interest and
6. the declaration of eradication areas in which testing and slaughter of reactors will be compulsory.

Some problem areas, notably manpower, funds and technical knowhow are identified and discussed, as is the role of the private practitioner in the Scheme.

A very short summary of progress to date is presented along with statistical detail.

Optimale benutting van ons land se beperkte hulpbronne vereis onder andere maksimale reproduktiewe doeltreffendheid in ons beesbevolking. 'n Nasionale brucellose insidens soos ons s'n (ongeveer 6 %) is onversoenbaar met doeltreffende reproduksie. Dit is nie om dowe neute dat brucelloses-uitroeiing die hoogste prioriteit geniet van veeartsenykundige owerhede in lande soos Kanada, Australië, Nieu-Seeland, die VSA en Groot-Brittanje nie. Afgesien van interne ekonomiese oorwegings, staan die internasionale handel op die punt waar nasionale vryheid van siektes soos brucellose 'n voorvereiste vir deelname sal wees.

By die beplanning van 'n skema vir die uitroeiing van brucellose moet faktore soos die nasionale insidens en die arbeidsintensiwiteit van toetsing deeglik in berekening gebring word. Hierbenewens is daar nie 'n enkele diagnostiese toets wat aan al die vereistes voldoen nie en die voorbehoedende entstof veroorsaak vals positiewe reaksies. Wanneer die simptoomlose aard van die besmetting, lang inkubasieperiode en die graad van aansteeklikheid (vir dragtige diere) nog bygevoeg word, is dit duidelik dat so 'n skema nie net baie duurder nie. maar ook meer veeleisend sal wees as wat die geval met beestuberkulose is.

**1.** Tot 1966, toe die Afdeling Veeartsenydiens die eerste voorleggings vir die instelling van 'n skema aan die Departementshoof gemaak het, was brucellosesbestryding beperk tot die vrywillige gebruik van S19 entstof en ad hoc optrede in besmette kuddes.

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Die entstof is hoofsaaklik voorgeskryf vir gebruik in verskalwers maar kon ook vir die beskerming van volwasse beeste gebruik word. Die skema, wat in 1968 deur die Minister van Landbou aangekondig is, sou op die volgende bene staan:

### 1.1 Verpligte inenting van verskalwers

Voorlopig was die ouderdomsgrense 4 en 10 maande. Die plan was om dit later na 6 en 8 maande te vernou. Amptelike inentings sou so vêr moontlik (gratis) deur die Afdeling se Veeinspeksiekorps gedoen word. Die onus om toe te sien dat sy kalwers geënt word, is egter op die eienaar geplaas. Entstof is nie gratis voorsien vir "private" inentings nie.

Verskalwers wat amptelik geënt was sou 'n driehoekige oormerk kry as bewys van inenting, of, in die geval van identifiseerbare diere, 'n amptelike entingssertifikaat.

### 1.2 Toedlening van entstof aan diere ouer as 10 maande

is onderhewig gemaak aan die skriftelike goedkeuring van 'n Staatsveearts. Sodanige goedkeuring is gewoonlik slegs vir besmette en bedreigde kuddes gegee en op voorwaarde dat die diere voor inenting gebloei is. Op grond van die uitslag van toetse op hierdie bloedmonsters kon "negatiewe" diere 'n omgekeerde oormerk kry as bewys van hulle brucellose-status tydens inenting.

### 1.3 Identifisering van reageerders of potensieël besmette diere

deur middel van die buisagglutinasietoets (BAT) op serummonsters en die melkringtoets (MRT) op melkmonsters. Laasgenoemde toets sou eintlik 'n siftingstoets



0038-2809/80/02/0078-0079

wees om moontlik besmette kuddes uit te wys. Diere van hierdie kuddes sou dan individueel serologies getoets word.

### 1.4 Brandmerk van reageerders

Aangesien onbeheerde inenting van volwasse diere tot 1968 plaasgevind het is voorgestel dat 'n moratorium van 2 jaar op die brandmerk van reageerders verklaar word. Hierna sou alle diere wat positief op die BAT reageer, met die letter "B" regs op die nek gebrand word. Die idee was om die boeregemeenskap op hulle hoede te stel teen sulke diere en dit moeilik te maak vir gewetenlose mense om hierdie "lopende tydbomme" aan niksvermoedende kopers af te smeer.

### 1.5 Akkreditering van kuddes

Die daarstelling van 'n vervangingsbron van brucellose-vry diere is as 'n belangrike faset van die skema gesien. Eienaars van kuddes sou op vrywillige basis aansoek om akkreditasie kon doen.

### 1.6 Afhangende van vordering, sou daar mettertyd uitroeilingsgebiede verklaar word

In hierdie gebiede sou alle diere gëdentifiseer moes wees, met streng toevoerbeheer. Alle bewegings sou onderhewig wees aan vooraftoetsing tensy die diere van 'n geakkrediteerde kudde kom. Monsters sou van alle volwasse koeie of verse by abattoirs geneem word en besendings melk sou gereeld met behulp van die MRT gemonitor word.

Die toediening van S19-entstof sou in hierdie gebiede verbied word om entstofreageerders sodoende mettertyd uit te skakel. Een of 2 jaar na hierdie verbod geldig geword het sou uitslagting van reageerders met betaling van vergoeding (60 % van markwaarde) in hierdie gebiede begin.

Om verskeie redes is daar tot 1976 nie veel vordering met hierdie skema gemaak nie. Geen fondse is byvoorbeeld ooit spesifiek vir brucellose bewillig nie. Mannekrag-probleme was die hoofrede waarom daar nooit veel meer as 60 % van die jaarlikse verskalweroes geënt kon word nie. Relatief min serologiese monsters is getoets en toetsmetodes was nie eenvormig nie. Groot probleme het onstaan omdat die vierde fase, naamlik brandmerk van reageerders, nooit ingestel is nie.

Teen 1976 het dit duidelik geword dat die identifisering van reageerders sonder om hulle permanent te merk en hul beweging te probeer beperk kontraproduktief was. Heelwat van hierdie reageerders het gedurig van eienaar verwissel en die besmetting is in werklikheid versprei.

**2.** Hoewel daar geen betroubare statistiek in 1966 of 1976 was nie het dit teen laasgenoemde datum geblyk dat die nasionale voorkoms van brucellose nie besig was om af te neem nie. Sommige werkers was selfs oortuig dat die teendeel waar was. Dit was duidelik nodig dat nuwe lewe in brucellose-bestryding geblaas sou moes word.

**2.1** Teen die einde van 1976 het die Afdeling bekend gemaak dat daar begin kon word met die akkreditering van kuddes. Eienaars wat (vrywillig) aansoek om toelating tot die skema gedoen het, moes 'n ooreenkomsvorm teken (CA1) waarin hulle onder andere toestem dat reageerders gebrand word en onderneem om hulle slegs vir slagdoeleindes te verkoop.

**2.2** Kort hierna is alle brucellosetoetse onderhewig gemaak aan die ondertekening van 'n ooreenkomsvorm CA1 soos hierbo en CA2 vir toetse wat nie met akkreditasie verband gehou het nie. Vorm CA2 bepaal in werklikheid slegs dat alle reageerders gebrand sal word. Van die Staat se kant is alle brucellosetoetse sedert 1-7-1977 gratis gedoen by staatsveterinêre laboratoria.

**2.3** Ontwikkelings sedert 1968 op die gebied van serologiese diagnostiek het wysiging van voorgeskrewe toetsmetodes genoodsaak. Daar is besluit om te standaardiseer op die Rose-Bengal plaattoets (RBT) vir siftingsdoeleindes met die Komplementbindingstoets (KBT) as beslissende toets. Die MRT sou sy plek as siftingstoets behou en kon ook aangewend word vir roetine toetsing in geakkrediteerde kuddes.

**3.** Aangesien daar nog steeds geen staatsfondse spesifiek vir brucellusebestryding toegeken was nie, is die gewysigde skema in Januarie 1979 in sy geheel aan die departement voorgelê. Beginselgoedkeuring is in April 1979 verkry vir die voortsetting en uitbouing van aktiwiteite en gedurende Januarie 1980 is die Afdeling in kennis gestel dat 'n doelwit vir 'n brucellose-skema geskep is en dat fondse vanaf die 1980/81 belastingjaar toegeken sal word.

**4.** Voorgaande paragrawe het die agtergrond tot die huidige posisie kortliks uitgespel. Voortaan sal daar meer doelgerig en volgens vaste plan as volg voortgebou word:

### 4.1 Verskalfinenting

met S19 sal steeds 'n hoeksteen van die skema bly. Daar sal gepoog word om S19-entstof van die oop mark te onttrek. Dit sal hopelik binnekort gratis aan boere en veeartse verskaf word by staatsveeartskantore; aan boere volgens die grootte van elkeen se kudde en aan veeartse vir gebruik in spesifieke kuddes. Die ouderdomsgrens sal vernou word van tussen 3 en 11 maande na tussen 3 en 7 maande.

### 4.2 Enting van volwasse koeie op selektiewe basis

(dit wil sê heelkudde-inenting). Onlangse intensiewe ondersoeke in die VSA het bewys dat hierdie maatreël epidemiologies en ekonomies geregverdig is. Deur gebruik te maak van 'n laer dosis word entstofreaksies tot 4 na 6 maande beperk. Die Navorsingsinstituut vir Veeartsenykunde te Onderstepoort is versoek om die moontlikheid van die vervaardiging van 'n spesiale S19-entstof vir volwasse enting te ondersoek. In die tussentyd sal Onderstepoort se S19-entstof in bedreigde kuddes gebruik word om aborsies te beperk en immuniteit te verhoog. Hierdie maatreël bly steeds onderhewig aan goedkeuring deur 'n staatsveearts.

Eienaars en hul veeartse moet duidelik verstaan dat dit 'n verkwisting van tyd is om volwasse-geënte kuddes binne die eerste jaar of twee weer te begin toets. Vertolking van laboratoriumuitslae is 'n onbegonne taak.

### 4.3 Identifisering van reageerders

4.3.1 Alle pogings word aangewend om serologiese toetsmetodes te standaardiseer. Die Rose-Bengal-toets sal by die NIV en al die Afdeling se laboratoria gedoen word sowel as by sekere staatsveeartskantore. Die komplementbindingstoets, daarenteen, sal slegs by 'n paar groter laboratoria gedoen word. Huidiglik is dit die NIV, Allerton, Stellenbosch, Middelburg (KP), Grahamstown en Kroonstad.

**4.3.2** Insgelyks sal daar op 7 mℓ ge-evakueerde glasbuise gestandaardiseer word vir die neem van bloedmonsters. 'n Finale besluit met betrekking tot die gebruik van bewaarmiddels in hierdie buise moet nog geneem word. Buise sal waarskynlik in skuimplastiekhouers voorsien word vir ordelike en veilige versending van monsters – in eenhede van 25. Buise en naalde word gratis aan meewerkers verskaf.

**4.3.3** Monsters wat deur boere of privaat veeartse ingestuur word moet vergesel wees van een kopie van ooreenkoms CA1 of CA2.

Alle monsters moet onder dekking van dekbrief CA3 en

monstervorm CA 5, na laboratoria gestuur word. Vorms is by laboratoria en staatsveeartskantore te kry.

Resultate word tot dusver nog alles deur die betrokke staatsveeartskantoor gekanaliseer. In die toekoms sal geakkrediteerde privaatveeartse hul uitslae waarskynlik direk van laboratoria kry. Die laboratorium voltooi slegs die toepaslike kolom op CA5 – met ander woorde RBT neg. of RBT pos., KBT x eenhede en pos dekbrief CA4 plus monstervorm CA5. Vertolking van hierdie titers is die verantwoordelikheid van die betrokke staatsveearts (geakkrediteerde privaatveearts).

**4.3.4** Dit spreek vanself dat diere waarvan monsters ingedien word, identifiseerbaar moet wees. Oorplaatjies sal teen staatskoste vir interim en geakkrediteerde kuddes verskaf word. Eienaars van kuddes wat vir ander doeleindes getoets word sal self vir die koste van identifikasiemaatreëls moet instaan.

### 4.4 Brandmerk van reageerders

Soos reeds vermeld, word alle reageerders sedert 1-7-1977 gebrandmerk. Die oorspronklike voorgestelde letter "B" is vir praktiese redes vervang met 'n "C". Tot dusver is die taak om reageerders te brandmerk slegs aan beamptes opgedra. Uiteindelik sal geakkrediteerde privaat veeartse hiermee behulpsaam wees. Die sukses van hierdie maatreël hang van die deeglikheid van die brandmerk self – dit moet 'n duidelike permanente brand wees – en die bekendheid van die publiek met die brandmerk af. Afslaers behoort voornemende kopers se aandag daarop te vestig.

Om verwarring in die boeregemeenskap te voorkom is daar 'n verbod op die hertoetsing van gebrandmerkte diere geplaas.

Hoewel eienaars wat die CA1 ooreenkoms met die Staat aangegaan het, kontraktueel en moreel verplig is om reageerders slegs vir slagdoeleindes te verkoop, kan spesiale vergunning aan kopers gegee word om hierdie diere te koop. Sodoende kan waardevolle teelmateriaal behoue bly – die voorwaarde is dat kopers ten volle ingelig is en besef dat hulle 'n risiko loop.

### 4.5 Akkreditasie van kuddes

Vanweë die epidemiologiese aard van brucellose moet kuddes streng gekeur word vir akkreditasie. Kuddebestuur, omheinings en geriewe moet baie goed wees. Dit mag selfs nodig wees om die siektestatus en bestuursvlak van nabygeleë buurplase in ag te neem. Keuring sal in alle gevalle deur 'n veearts gedoen moet word.

Aangesien die primêre doel met die akkreditering van kuddes die voorsiening van 'n vervangingsbron is, sal teelkuddes van redelike grootte voorkeur kry van staatskant. Dit verhoed nie eienaars van klein teelkuddes en kommersiële melk- of vleisprodusende om hul kuddes op eie koste te laat akkrediteer nie.

Afgesien van genoemde streng keuring, is die voorwaardes vir akkreditasie ten minste 2 negatiewe kuddetoetse (serologies) 12 maande uitmekaar. Alle aanteeldiere 18 maande en ouer word getoets. Toevoegings tot kuddes moet ten minste 2 negatiewe toetse, 60 dae uitmekaar, ondergaan. Hulle moet in isolasie gehou word vir die 60 dae. Koeie en verse wat meer as 4 maande dragtig is mag nie by die kudde aansluit voordat 'n negatiewe toets 4 weke na kalwing gedoen is nie.

Geakkrediteerde kuddes word jaarliks hertoets soos vir akkreditasie. As alternatief kan melkkuddes elke 4 maande op kuddebasis aan die MRT onderwerp word.

Eienaars van geakkrediteerde en interimkuddes is verplig om alle aborsies en vroeë geboortes aan te meld en, waar enigsins moontlik, monsters vir diagnostiese doeleindes in te dien.

### 4.6 Verklaring van uitroeiingsgebiede

Soos in die oorspronklike voorlegging uitgespel is dit steeds deel van die plan. Die tydstip wanneer dié fase in werking gestel sal word moet nog bepaal word. Basies sal dit afhang van die persentasie kuddes wat reeds in die betrokke gebied geakkrediteer en/of diagnosties getoets is en die voorkoms van besmetting in die gebied.

### 4.7 Uitslag van reageerders

Tot op die huidige stadium word reageerders vrywillig en op eie koste deur eienaars uitgeskot. Sodra uitroeiingsgebiede verklaar en boere verplig word om hul diere te laat toets en uitslag sal een of ander metode van vergoeding uitgewerk moet word. Die feit dat reageerders "gemaak" kan word deur die toediening van entstof, veroorsaak dat hierdie aspek veel meer gekompliseerd is as met die tuberkuloseskema. Geen finaliteit is nog hieroor bereik nie.

### 4.8 Aansporingsbonusse

Tot dusvêr was die landswye belangstelling in die skema sulks dat die aanvraag na deelname nie bevredig kon word nie. Een of ander tyd sal dit egter nodig word om die belangstelling te stimuleer om seker te maak dat die skema nie momentum verloor nie. Die beginsel om 'n bonus aan melkprodusente met brucellose-vry kuddes uit Staatsfondse te betaal, soos in Engeland, klink goed. Vir vleiskuddes word 'n kontantbedrag per teelkoei jaarliks uitbetaal.

Die belangrikste voorwaarde vir die aanvaarding van die beginsel sal die beskikbaarheid van dienste aan alle beesboere wees.

Indien hierdie bonusse aantreklik genoeg is kan dit selfs oorweeg word om weg te doen met vergoeding vir die uitslag van reageerders. Hierdie aspek geniet ook nog aandag.

## 5. Knelpunte

Daar is reeds melding gemaak van knelpunte wat vordering tussen 1968 en 1976 belemmer het. Baie van die probleme bestaan steeds. Vir die sukses van die skema is dit nodig dat die knelpunte geïdentifiseer en planne beraam word om hulle te omseil.

### 5.1 Mannekrag

Soos reeds genoem, is brucellose-beheer arbeidsintensief. In die laboratorium kan toetse tot groot hoogte reeds geoutomatiseer word maar monsterversameling bly steeds 'n probleem.

#### 5.1.1 Laboratoriumpersoneel

Huidig beskikbare geriewe en personeel sal uiters 'n half miljoen monsters per jaar kan hanteer. Vooruitbeplanning mik na 1 miljoen binne 4 jaar. Hoewel dit nie 'n verdubbeling van werkkragte impliseer nie is dit duidelik dat ekstra werkers – hoofsaaklik tegniese assistente – benodig sal word. Die ideaal is dat elke staatsveeartsarea se monsters in die area aan die siftingstoets (RBT) onderwerp moet word, maar die personeel hiervoor sal moeilik binne die eerste 5 jaar bekom word. Laboratoriumdiagnostiek sal myns insiens die Staat se verantwoordelikheid bly.

#### 5.1.2 Veldwerkers

Die groot knelpunt lê hier. Tans word monsters deur veeinspekteurs op 'n deeltydse basis geneem, sowel as deur staatsveeartse en privaat praktisyns (sonder staatsvergoeding). In die afgelope 8 maande het uitbrekings van bek-en-klouseer en die nasionale skaapbrandsiektekampanje die veeinspekteurs se aandeel so gekortwiek dat daar in plaas van 'n toename in toetse, 'n merkbare afname was – naamlik van 'n maandelikse gemiddeld van ± 22 000 vir die jaar tot 30-6-1979 na ± 16 000 vir die 6 maande tot 31-12-1979.

Die ondervinding in die VSA het geleer dat dit fataal is om die boeregemeenskap vir 'n skema te motiveer, daarna briek aan te draai en dan weer belangstelling te probeer wek. Dit is met ander woorde essensieel dat die Staat sy verpligtinge teenoor deelnemers aan die skema nakom.

Om dit te kan doen moet 'n aantal veeinspekteurs van roetine veeinspeksiewerk onthef word om voltyds brucellose- (en tuberkulose)-werk te doen en/of meer veeinspekteurs moet aangestel word en/of die hulp van die privaatsektor moet ingeroep word. In die kort termyn lê die oplossing klaarblyklik by die eerste en/of die laaste alternatief.

Die dienste van die privaatpraktisyn sal aangewend kan word oor die hele spektrum van veldaktiwiteite van die skema. Vir sovêr dit keuring van kuddes, advies aan boere en vertolking van laboratoriumresultate aangaan, sal dié kollegas se hulp aan die staatsveearts met verloop van tyd onontbeerlik word. In beginsel is die inskakeling van privaatveeartse by die skema, met vergoeding min of meer soos by die tuberkuloseskema, reeds goedgekeur. In die 1979-voorlegging is egter nie voorsien dat die privaatsektor só gou betrek sou word nie. Daar is derhalwe nie daarvoor begroot nie en spesiale goedkeuring sal nou vir die bespoediging van dié fase verkry moet word.

### 5.2 Fondse

Die Staat, by monde van die Afdeling Veeartsenydiens, het reeds aanvaar dat die bestryding en uitroeiing van brucellose binne sy wetlike opdragte val. Soos reeds vermeld is fondse dan ook reeds vir die doel bewillig.

Uit die aard van die saak sal individuele boere finansiële opofferings moet maak, veral in die vrywillige fase, om van die siekte ontslae te raak. Op die lang termyn moet hierdie uitgawes egter as beleggings beskou word wat ongetwyfeld dividende sal afwerp.

Indien die idee van 'n aansporingsbonus aanvaarbaar is, ontstaan die geleentheid vir die bedryf as geheel om ook 'n bydrae te lewer. Dit word in die geval van die suiwelbedryf reeds toegepas deurdat 'n premie aan leweransiers van "kwaliteitsmelk" betaal word. 'n Soortgelyke bydrae ten opsigte van brucellose-vry melk kan tegelyk dien as stimulus vir die skema en om die Staat se las in dié opsig te verlig.

### 5.3 Tegniese knelpunte

kan verdeel word in universele probleme en plaaslike probleme. Universele probleme soos die soektog na 'n meer doeltreffende entstof, een enkele eenvoudige diagnostiese toets en selfs behandeling van (besmette) waardevolle teeldiere geniet wêreldwye aandag van navorsers. Hoewel dit nie beteken dat ons mag terugsit en toekyk nie, geniet dié aspek nie plaaslik 'n baie hoë prioriteit nie.

Plaaslike knelpunte wentel tans nog om die standaardisering van (a) monsterneming, met inbegrip van houers, bewaarmiddel, versending ens. en (b) die twee standaardtoetse, naamlik RBT an KBT met al die reagense wat daarvoor gebruik word. Goeie vordering is reeds gemaak en daar is alle hoop op sukses.

## 6. Vordering tot dusver gemaak

Ten spyte van bogenoemde probleme en knelpunte was daar gedurende die afgelope 3 jaar bemoedigende vordering. Ons laboratoriumdiens se toetsvermoë is tans, soos reeds genoem, ongeveer 'n half miljoen monsters per jaar. Op die standaardiseringsfront was vordering stadig maar beslis daar. S19 verskalf inentings op nasionale basis is ongeveer staties en nuwe inisiatiewe op dié front is broodnodig, soos reeds genoem.

Statistiek met betrekking tot brucellose bestryding tot 30-6-1979 word in Figuur 1 en 2 en die meegaande tabel weergegee.

Die toestemming van die Direkteur van Veeartsenydiens om hierdie artikel te publiseer word met dank erken.

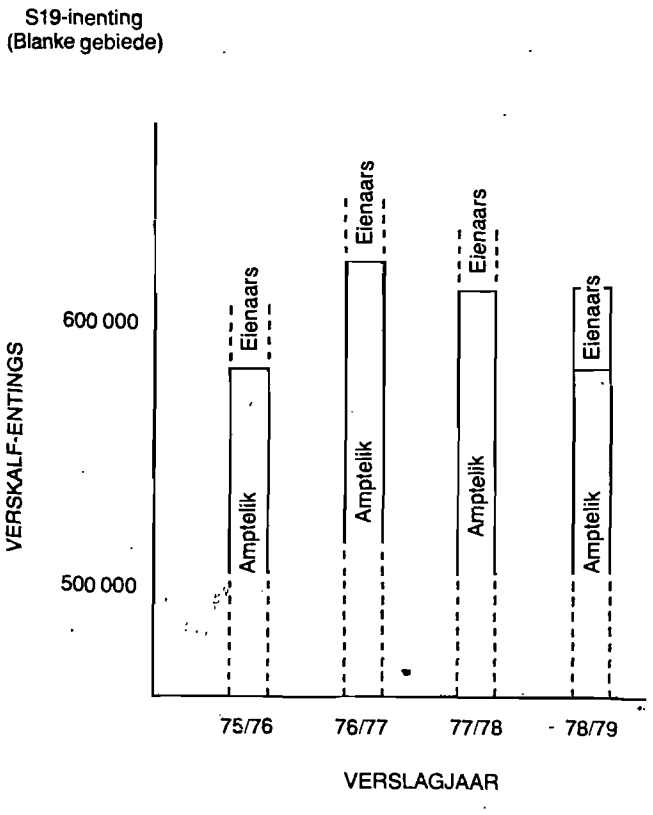


Fig. 1 Beesbrucellose

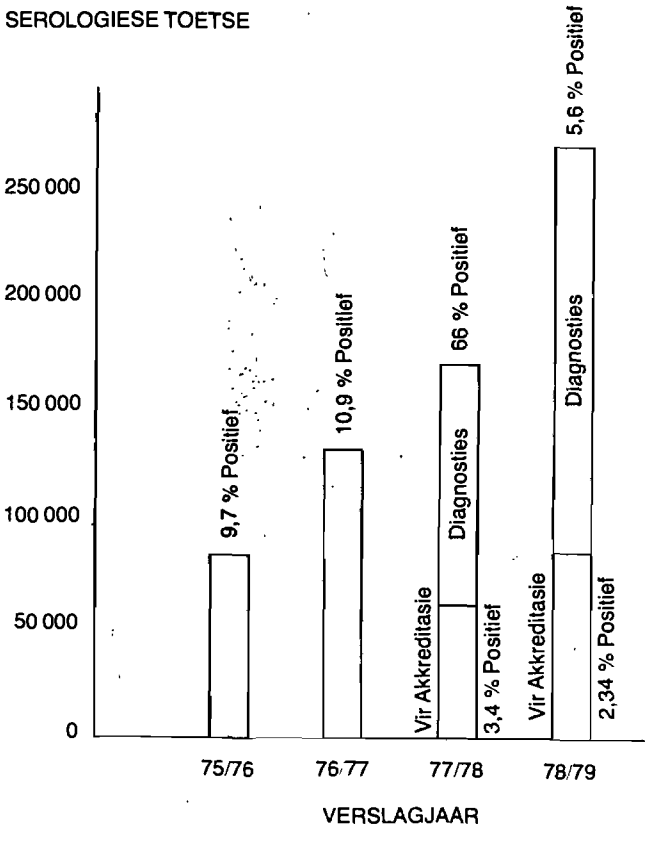


Fig. 2 Beesbrucellose

Table 1: BEESBRUCELLOSE : OPGAWE

(1) INENTINGS EN TOETSE 19780701 tot 19790630

| Streek | Inentings Deur eienaar | Inentings Amptelik Verse | Inentings Amptelik Koeie | Toetse vir Akkreditasie Kuddes | Pos | Aantal Beeste | Pos | Verdag | Diagnostiese Toetse Kuddes | Pos | Aantal Beeste | Pos | Verdag |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Transvaal | | 114 986 | 1 402 | 25 | 14 | 2 565 | 148 | 47 | 421 | 205 | 21 816 | 1 931 | 105 |
| N & O Tvl | 6 371 | 79 971 | | 28 | 12 | 3 876 | 67 | 10 | 394 | 191 | 32 739 | 1 146 | 97 |
| Hoëveld | 41 273 | 66 997 | 753 | 28 | 7 | 3 375 | 34 | 2 | 755 | 432 | 40 998 | 3 719 | 295 |
| OVS | | 86 340 | 420 | 12 | 6 | 1 078 | 43 | 19 | 127 | 83 | 7 087 | 677 | 318 |
| Natal | | 155 055 | 2 470 | 126 | 19 | 14 405 | 56 | 174 | 827 | 299 | 63 641 | 2 045 | 1 082 |
| Oos-Kaap & K | 1 907 | 50 082 | | 129 | 49 | 12 746 | 247 | 143 | 208 | 31 | 5 945 | 144 | 18 |
| Wes-Kaap | 2 726 | 22 012 | 1 013 | 700 | 192 | 46 980 | 1 396 | 318 | 224 | 64 | 11 976 | 725 | 118 |
| Groot totaal vir verslagjaar | 52 277 | 575 443 | 6 058 | 1 048 | 299 | 85 025 | 1 991 | 713 | 2 956 | 1 305 | 184 202 | 10 387 | 2 033 |

(2) TOTALE GETAL KUDDES EN BEESTE INGESKAKEL BY AKKREDITASIE-FASE VAN SKEMA

| | Geakkrediteer | Interim |
|---|---|---|
| Kuddes | 88 | 491 |
| Beeste | 6 469 | 48 476 |

## ISOLATION AND CHARACTERIZATION OF ANTIBODIES TO *CLOSTRIDIUM PERFRINGENS* EPSILON TOXIN FROM HYPERIMMUNE HORSE SERUM

R.W. WORTHINGTON and MARIA S.G. MÜLDERS

ABSTRACT: Worthington R.W. & Mülders Maria S.G. 1979. The isolation and characterization of antibodies to *Clostridium perfringens* epsilon toxin from hyperimmune horse serum. *Onderstepoort Journal of Veterinary Research*, 46. 121-124 (1979).

Antibodies against epsilon toxin were isolated from hyperimmune horse serum by affinity chromatography. Purified epsilon prototoxin covalently bound to Affigel 202 was used as immunosorbent, and antibodies were eluted with 6,0 M guanidine chloride. In a single run 80 mg of antibody could be recovered from a 20 mℓ column of immunosorbent. The antibody was shown to belong to the IgG(T) class of immunoglobulins.

## SUBCUTANEOUS AND PULMONARY EMPHYSEMA AS COMPLICATIONS OF BOVINE EPHEMERAL FEVER

A. THEODORIDIS and J.A.W. COETZER
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ABSTRACT: Theodoridis A. & Coetzer J.A.W. 1979. Subcutaneous and pulmonary emhysema as complications of bovine ephemeral fever. *Onderstepoort Journal of Veterinary Research*, 46, 125-127 (1979).

Subcutaneous and pulmonary emphysema was observed in some cattle on farms on which outbreaks of bovine ephemeral fever (BEF) occurred. BEF virus was isolated in baby hamsters from one of the cases and cattle were injected with blood from this animal. Although the experimental animals developed typical BEF symptoms, no signs of emphysema could be detected by clinical and pathological examinations.

The histopathological changes in the skeletal muscle and synovial membranes of the natural case resembled those of BEF described by Basson, Pienaar & Van der Westhuizen (1970). The lumina of the terminal and respiratory bronchiles in the lungs were obliterated by cellular debris and the muscular portion of some of those bronchioles was necrotic. The possible pathogenesis of pulmonary emphysema is discussed.

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4. Gebruik die 'Head-Count' maethouer om genoegsame dipkonsentraat voor elke dipping, volgens getal beeste wat met vorige dipping gedip was, af te meet.
5. Indien dipbak oorstroom word, verwyder oortollige water versigtig vanaf oppervlekte van die dipbak by die uitgang (treppies) totdat inhoud van dipbak weer die "VOLMERK" bereik. Voeg dipkonsentraat met maethouer, volgens getal beeste met vorige dipping gedip, by.

NUWE OF SKOONGEMAAKTE DIPBAK:

6. Wanneer 'n dipbak skoongemaak is, vul die dipbak met skoonwater tot op gekalibreerde "VOLMERK" en voeg 1 liter Supamix D.F.F.* of Delnav D.F.F.* vir elke 2200 liter water in dipbak by. Dip en tel die beeste. Voeg dipkonsentraat met maethouer volgens dié getal beeste voor volgende dipping by en volg dié prosedure met die daaropvolgende dippings.

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* SA Patent Nr 68/5864 SWA Patent Nr 69/115


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**CONGRESS PAPER** **REFERAAT**

# VETERINARY MANAGEMENT OF ENDURANCE TRAIL RIDES

MURRAY E. FOWLER*

## INTRODUCTION

Veterinary surgeons may become involved in many different types of trail rides. The pleasure ride is usually a 8–24 km ride of family, friends or a riding club. The gait is a walk or trot. There is a lot of time for fun and relaxation. Horses are neither monitored nor checked for condition. Even under such mild stress conditions, overweight and under-used horses will have trouble and require veterinary assistance.

The competitive trail ride may last from 1–3 d with distances of 48–64 km ridden in 6–7 h. Horses move at walk, trot and occasional canter. In rides in the North American trail ride conference, the horse is judged on the basis of soundness – 40 %, condition – 40 %, way of going – 15 %, and manners – 15 %. A veterinary surgeon is usually one member of the judging team. Although the pace is not intense, adverse weather conditions can develop that will cause concern.

Endurance rides are a little tougher. The rides usually last 1 or at the most, 2 d. The distance is 80–160 km. The time is variable, based on the terrain but speed is important. The horse and rider must traverse an extended distance within a fixed time limit. On the Tevis Cup ride, the front running horses will travel 160 km in 13 h of riding time at an average pace of 12 km/h and all finishers must complete the ride within 24 h. Condition is of vital importance. It is crucial that veterinary surgeons be an integral part of the management of these rides.

A few rides are being set up in the United States that are to all intent and purpose, a horse race of 80 km or more. Veterinary surgeons are asked to serve on these rides to prevent abuse of the horses. I, personally, don't like to work these rides because it is difficult to establish and maintain control.

## PRERIDE UNDERSTANDING

A veterinary surgeon (VS) will be invited to serve as the chairman of the veterinary examining committee. Depending upon the number of horses and the terrain, anywhere from 3–15 veterinary surgeons may be required to man the various checkpoints.

Hopefully, the VS will be involved in the policy-making process for the ride and be in a position to incorporate sound management practice that will aid horses. The VS should have a firm and even written understanding of what is expected of the committee and more particularly, the authority delegated to the veterinary committee. I personally would not work a ride unless the veterinary committee has absolute control as to whether a horse starts the ride or is allowed to go on at any checkpoint.

One must obviously check on such things as preride check-in times, accommodation (which can vary tremendously), transportation, communication, recorder help, renumeration, trail markings, maps and companions for juvenile contestants. Many of the veteran trail ride VS's have ridden the terrain or have even competed in the rides they work. This gives the VS a special feeling for the conditions to which the horse and rider are subjected.

Important considerations are the location and spacing of the checkpoints. Long established rides like the Tevis Cup ride in California already have the checkpoints set up. The first checkpoint is about 51 km from the start. If the terrain were more rugged, perhaps a shorter distance would be required. The middle section of the Tevis Cup ride is a tough stretch with a considerable amount of ascent and descent. Temperatures in the bottom of these canyons can reach 41°C in mid-afternoon when most horses go through them. There must be adequate space at the checkpoint for examination of the horses and opportunity for the horses to rest, be treated or transported out if required. Be sceptical about extremely hot, cold and/or windy locations.

Terrain may dictate the spacing but three 1-h rest stops should be a minimum on a 160 km ride.

## PRERIDE EXAMINATION OF THE HORSES

All horses should have been given thorough veterinary examinations during the training period. Regular worming and other preventive care should be standard. All horses must be examined by the veterinary committee the day prior to the ride.

Inexperienced VS's will be surprised at the array of shapes, sizes and breeds of horses competing in endurance rides. Arabian types or Arabian crosses tend to be the top endurance horses in the US but I've seen every breed of horse plus mules compete successfully. One horse that completed 10 Tevis Cup rides and won 2 of them was a nondescript cow pony from a dude ranch rent string.

The ideal endurance horse has a broad chest, lithe supple muscling, a long sloping pastern, strong smooth trot and is responsive to the bit. Excess weight is a detriment. Some would designate endurance horses as thin but no more so than a marathon runner.

Size has little to do with success. The mare that I rode was an Arab cross with a mass of 387 kg at the beginning of training. At the time of the ride this had dropped to 351 kg. She carried 113 kg of rider and tack or almost $^1/_3$ of her own mass.

You might be interested in some comparisons of training schedules of endurance horse and other athlet-

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es. An endurance horse will train at 80–240 km/week over a period of 6–8 months. Intermittently, the horse will work 80 km in a day.

A Thoroughbred or quarter horse racer will only gallop 2 km/d and have a work of 1 km each week. A standardbred in the US will trot 2–4 km/d with 24 km maximum/week.

Human marathoners are thought to have the most vigorous training schedule. They train 80–128 km/wk with a maximum up to 32 km/day. This is done for 3 months prior to a run. The trail horse doubles the marathoner.

At the preride examination one can get carried away with minutiae. The crux of the examination should include an evaluation of the horse's general appearance and condition, gross abnormalities of feet and legs, an evaluation of the cardiovascular system, a check for vision and age and observation of the horse in motion.

Causes for elimination at the preride examination include lameness, fever, serious wounds, fresh and active saddle sores and total lack of condition, either thinness or obesity.

The VS must be cautious about being led into making a definite diagnosis as to the cause of lameness. That shouldn't and usually can't be done on a cursory inspection. If the horse is lame it must not be allowed to start the ride. Otherwise the horse will plague you throughout the ride as to whether things are getting worse, etc.

Some horses will be stiff if trailered for some distance. If the preride check is the day previous to the ride, make a last minute check in the morning for any evidence of colicy horses, rope burns, lacerations, kicks or cool lamenesses. Some riders will disguise a slightly lame horse for the precheck by warming it up before presenting the horse for inspection.

## BRIEFING RIDERS AND VETERINARY COMMITTEE MEMBERS

After all the horses have been checked in, it is important to have all the riders and management gather to discuss rules and regulations and answer questions. It is worthwhile for a member of the veterinary committee to explain some of the special things they'll be looking for in evaluating horses. Foreknowledge of problem areas in terrain or weather can prevent major catastrophies. Let the riders know what is expected of them and their horses.

These rides are a lot of fun and even though grueling for horses, riders, crews and veterinary surgeons, we keep coming back. There is a lot of satisfaction in getting 300 horses over a mountain a distance of 160 km without major mishaps.

The veterinary team should also get together so that evaluation can be consistent. Schedules should be distributed so all will know where they should be and when. Once the ride begins, members of the team may not see one another until after the ride. I distribute the ground rules for evaluation that are agreed upon by the team.

## CHECKPOINT EVALUATION

Horses sould be timed in at each checkpoint and the heart and respiration rates noted immediately. Notice the presence of depression, dehydration, tonus of the anal sphincter and gait. Then allow the horse to cool and rest.

In 15–45 mins (depending upon the ride) the horse is called back for a recheck of heart and respiratory rates. Check the saddle and cinch area and observe the horse at a walk or trot.

Lameness is an automatic disqualification. Other factors must be weighed and a decision made as to whether the horse should go on. Each veterinary examining committee may set the criteria for passing a recheck but many designate that the pulse must be below 70 and respiration below 40. Other unsatisfactory cardiopulmonary responses include a respiratory rate that is faster than the heart rate. irregular heart beats and thumps (SDF).

Some members of the veterinary team must take the time to stand back and get an overall impression of the horse. It is easy to get all the objective measurements but clinical judgement is still extremely important.

A word of caution. Horses may have elevated heart and respiratory rates at the recheck for reasons other than exhaustion. These reasons include failure of the rider or his crew to let the horse rest or the horse drinking cold water, colic and excitement.

The most pressing need of the endurance trail ride VS is to have objective criteria upon which to assess the condition of a horse. There are telltale clinical signs that alert the astute observer. Nonetheless, as you send a horse and rider out from a checkpoint, you would like to have measurable criteria to make sure the horse can make it to the next point.

Although we try to get as much objective data as possible, there should be an agreement as to subjective factors which will be grounds for disqualification. At least two VS's should concur on whether or not a horse should go on or be eliminated.

Research VS's working with us on our rides have amassed a good deal of laboratory data and have established normal and abnormal haematologic and blood chemistry patterns. The amenities of a good clinical laboratory are never available to be of use in on-the-spot evaluations on the ride. There are, however, some field tests that have been developed that could be of value – particularly in selecting a course of therapy for a given horse.

Packed cell volume (PCV), total plasma proteins (TPP) and hemoglobin (Hb) are all quite reliable in establishing the degree of dehydration. PCV's can be performed quickly using the microhematocrit technique if electrical power is available. Portable power generators are frequently used on endurance rides to provide lighting at night check points.

TPP's are easily run using a refractometer and Hb's are calculated using a haemoglobinometer. These field tests correlate with standard laboratory techniques well enough to make them useful in the field.

Hypochloraemia and hypocalcaemia are the two electrolyte alterations that are most likely to contribute to the exhaustion syndrome. Fortunately, fairly accurate field titration techniques have been developed (Carlson) that can provide on-the-spot assessments of these two electrolytes.

An assessment of the acid-base balance of the blood is very difficult yet crucial to proper evaluation of a horse for therapy. A horse that has been over-ridden at high speed may be acidotic and require sodium bicarbonate therapy. The more usual condition in the exhausted horse syndrome is that the horse has developed a mild metabolic alkalosis.

Carlson has found a good correlation between total plasma carbon dioxide levels and pH. Using a commercial apparatus, he can conduct a field test to measure total $CO_2$.

Other field tests for glucose, haemoglobinuria, myoglobinuria, bilirubinuria and others could possibly be used but have not proved particularly useful during the ride. Haematologic changes occur in all horses during endurance rides. The major response is that of fluid volume loss brought on by the need for the horse to cool itself by sweating. The preride PCV of conditioned Arabian type horses may be lower than that of Thoroughbreds conditioned for racing on a track. A PCV of 35 is normal. All horses on a ride will dehydrate. The PCV on fit horses will rise to 45 while those destined to become exhausted may rise above 55 denoting severe dehydration.

### FINAL EXAMINATION

Horses are examined, rested and rechecked at various points along the course of the ride including the finish. Some rides will have a special award for the horse making the distance in the best condition. This will require a reevaluation of the horses the day after the ride. Procedures similar to the checkpoint evaluation are carried out except that pulse and respiration are not considered.

Muscle soreness and foot soreness are almost sure to be present, so due consideration should be given to this.

The veterinary team will then retire to deliberate on the records kept of the horses during the ride and the final decision, in writing, should be given to the management.

## INFORMATION INLIGTING

### ACUPUNCTURE FOR LIVESTOCK

The first acupuncture anaesthesia demonstration on large animals ever to be given by Chinese veterinarians in the United States, will be staged at the International Stockmen's School being held this month (January 7–10) at Tucson, Arizona, according to Dr M.E. Ensminger, Director of the School.

The three famed specialists selected by the Ministry of Agriculture, People's Republic of China, for the historic acupuncture demonstration are: Dr Chen Cilin, Specialist in anatomy; Dr Peng Hongze, Specialist in surgery; and Dr Sun Yongcai, Specialist in Chinese traditional medicine.

According to Dr Ensminger, who has seen needle-induced anaesthesia used in China on both humans and farm animals, acupuncture has a history in China dating back 4 000 years. Medical doctors and veterinarians did not start using it as a pain-killer and anaesthesia for surgery until 1958, however. Since then, it has been used in thousands of operations on the heart, chest, abdomen, and limbs, and even in open-heart surgery. (Human patients feel no pain as a result of this method, and are fully conscious during surgery). "Animals will munch feed right after surgery", according to Dr Ensminger.

The needles are inserted into the body at predetermined points, and they may be manipulated gently by a twirling motion, either by hand or electrically. The surgical teams in China have achieved 90 to 95 per cent success with animals.

("Acupuncture for Livestock": The Drovers Journal, November 1, 1979, p. 48, Vance Publishing Company, Food & Agriculture Division, One Gateway Center, Kansas City, Kansas 66117)

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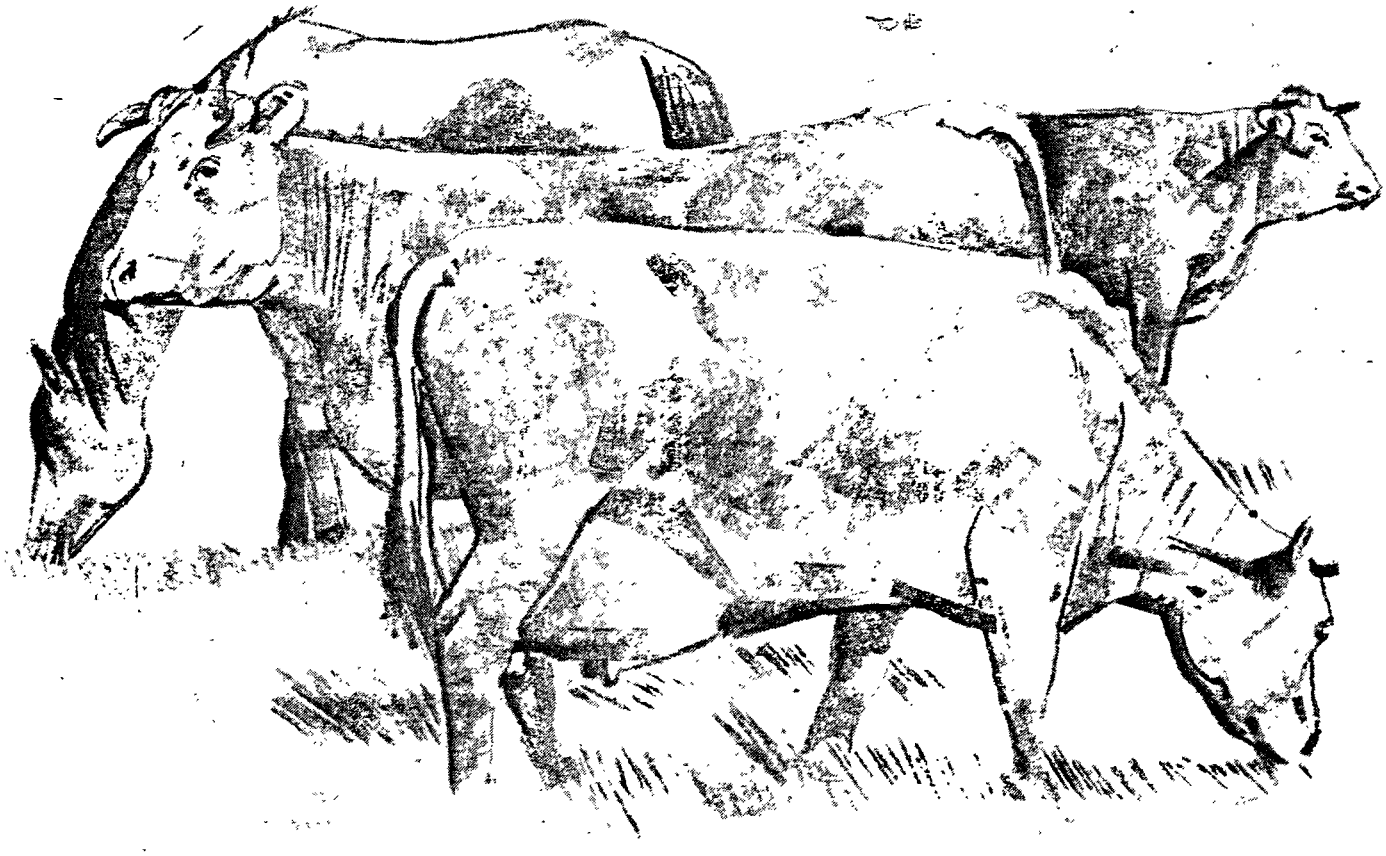

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# EXHAUSTED HORSE SYNDROME

MURRAY E. FOWLER*

The term "exhausted horse syndrome (EHS)" was coined to describe a complex metabolic disease occurring when horses are pushed beyond their endurance. The syndrome has no precise characterization because the initiating mechanism may vary with the set of conditions operating on a ride. The development of EHS will depend on the condition and training of the horse, the pace set by the rider, the terrain traversed and climatic factors such as temperature, wind, and humidity.

The dramatic increase in trail riding, especially endurance riding, has brought many veterinary surgeons into contact with exhausted horses. Previously the syndrome was seen only in horses used in military campaigns. Although other medical problems are seen in track race horses, track veterinary surgeons will not see the exhausted horse syndrome.

Three-day event horses sometimes become fatiqued but do not suffer the serious metabolic deficiencies of EHS horses.

## CLINICAL SIGNS

A composite picture will be presented but the individual horse may exhibit only a few of the signs. Following is a description of the severely affected horse.

The horse is depressed, the head is held low and movements are lethargic. The horse takes no interest in its surroundings. The ears are expressionless. Anorexia is typical and frequently there is no inclination to drink even though the horse is dehydrated. The cornea exhibits a glazed appearance. Grimaced facial muscles give an anxious expression that may progress to a painful expression if colic or muscle spasms accompany the syndrome.

Body temperature is usually elevated and may reach 41°C (106°F). The horse doesn't cool properly. Commonly, the temperature is higher at the recheck than when first arriving at a checkpoint. Rectal temperatures may be inaccurate in the exhausted horse. Frequently, the anal sphincter will lose tone and gape, allowing air to enter the rectum.

Many normal horses show some degree of anal dilatation upon arrival at a checkpoint but if the anus is pinched the sphincter constricts, closing the orifice. The exhausted horse is unresponsive to such stimulation.

Cardiovascular and respiratory systems are markedly affected by the exercise of endurance riding. Heart rate and respiratory rate are elevated. The degree depends upon the condition of the horse, the pace, the length of action and the amount of work performed in climbing or walking on soft footing. Heart rates of 150/min are not uncommon after a grueling climb. In 10–15 minutes the heart rate of the conditioned horse will drop below 60 whereas the exhausted horse may persist in showing a tachycardia and tachypnea.

The exhausted horse may have respiratory rate that is faster than the heart rate. Respiration under these circumstances is shallow and inefficient in respiratory exchange.

Additional cardiopulmonary signs may include synchronous diaphragmatic flutter (SDF), arrhythmias, murmurs and jugular pulses. Auscultation of the thorax may reveal moist rales and, in extreme cases, pulmonary edema may develop.

The difference between the exhausted horse and the normal horse is that the exhausted horse is incapable for a variety of reasons of re-establishing normal cardiopulmonary function.

Severe dehydration is the most consistent sign of EHS. Loss of skin elasticity, sunken eyeballs, dryness of the mouth and other mucous membranes reflect a 7–10 % loss of body weight as a result of the loss of 30–40 litres of fluid. Serious electrolyte imbalances and upset acid-base balances are associated with dehydration. Laboratory procedures are required to measure electrolytes. The description of the pathophysiology of EHS will bring out the significance of these alterations.

Muscular manifestations of EHS may include fatigue, trembling, spasm, stiffness, muscles painful to palpation and, in a few cases, tying-up. Tying-up, however, is usually a distinct entity.

EHS horses are prone to develop colic but colic may occur independently of EHS. When it accompanies EHS, colic is of the spasmodic type with diminished or absent borborygamus.

## PREDISPOSING FACTORS

Predisposing factors include all adverse environmental conditions, particularly terrain, wind, humidity and ambient temperature. The degree of training and conditioning of the horse has a lot of bearing on the potential development of EHS.

The rider plays a vital role. Some riders who are superb horse trainers can't cope with competition. They over-ride their horses continually if not monitored carefully.

## PATHOPHYSIOLOGY

The endurance horse must have all body systems in balance with one another. In the training process, the rider must be certain to condition all systems. The horse shouldn't be muscle conditioned to the exclusion of conditioning the respiratory or cardiovascular systems.

The basic physiological response to work and heat

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stress should be understood in order to cope with the exhausted horse syndrome.

When a horse begins to exercise, the body temperature rises and the horse thermoregulates by sweating. The degree of sweating will vary with the amount of work involved, the environmental temperature, and the humidity. At first, the fluid loss will come from the extracellular fluid but ultimately, the intracellular fluid pool will contribute to the fluid loss.

Carlson has extensively studied the electrolyte alterations in endurance horses. One should read his work to understand the intricasies of electrolyte shifts. He has found that in both normal horses and horses prone to exhaustion there is a hyponatraemia, hypokalaemia, hypochloraemia and hypocalcaemia. Dehydration exacerbates these deficiencies.

The source of loss of the electrolyte becomes evident when the composition of sweat is a known figure (Carlson). The quantity of fluid lost through evaporative cooling is tremendous (30–40 $\ell$). It is obvious that the quantity of electrolytes lost is significant also.

Haematological changes reflect a general stress response. There is a leukocytosis brought about by a significant neutrophilia. Lymphocytopenia and mild eosinopenia are also seen. Both normal and exhausted horses show a similar haemotologic response except that the exhausted horse usually has a marked left shift in the neutrophils. The mechanism for this shift and its significance are unknown.

If a horse's pace is extremely fast – over 600 meters/min (36 km/h), 22 mph) – the horse will go into anaerobic oxidation to supply energy needs. Lactic acid is one of the byproducts of anaerobic oxidation. The build-up of lactic acid causes metabolic acidosis. It is vital, however, that the veterinary surgeon be fully aware that anaerobic oxidation can persist for only from 5 to 10 min. At that point the animal must shift back to aerobic oxidation or slow down as muscles fatigue.

Well conditioned or properly ridden endurance horses rarely reach speeds that require anaerobic oxidation. Therefore, metabolic acidosis is rare. Rather, fluid and electrolyte shifts alter metabolism and the result is a mild metabolic alkalosis.

The parameters of the exhausted horse, taken initially at the checkpoint examination will be similar to those of a normal horse.

Both may arrive at a checkpoint with tachycardia, tachypnea and marked dehydration after traveling for 40–57 km (25–35 miles). The PCV would be elevated, as would the total plasma protein (TPP). The body temperature may be as high as 41°C (106°F).

A normal horse is capable of rehydrating itself when given access to water but it does not have the reserves of electrolytes upon which to draw. The deficit must be supplied orally. After rehydration both the normal and exhausted horse may come up for a recheck with even more significant electrolyte deficiencies than at the initial check.

There are variations in the exhausted horse syndrome depending upon the initial condition of the horse. Poorly conditioned horses will get into trouble more quickly and their metabolic problems be more intense. These horses are more likely to develop acidosis and various muscle problems. The more highly conditioned horse will take longer to develop the syndrome and there will probably be an alkalosis. Don't be misled!! Even the most highly conditioned horse can become exhausted under a given set of circumstances.

In summary, the exhausted horse syndrome is brought about by work and heat stress. Dehydration, electrolyte alteration and acid-base imbalance act as a complex metabolic upset producing the various signs already described.

## THERAPY

Rest, rehydration and electrolyte supplementation are the keys to recovery. There was a time when I felt that I had to intensively treat every exhausted horse. I have used steroids, vitamins, fluids and every miracle cure I could think of. Cortisol levels are already elevated from the stress of the ride; unless the horse is nominally adrenocortically exhausted, steroids will be of little help.

If the horse is drinking and will take electrolytes in the water, the effect wil be nearly as beneficial as administration of intravenous fluids. Obviously, if the horse is hyperthermic, shocked and refuses to drink, more drastic steps must be taken to rehydrate the horse. Forty to 50 litres of fluid may be required. Besides intravenous administration, one can also use gastric intubation and give fluid *per os*. Enemas are also effective, as fluid is absorbed from the colon.

## PREVENTION OF EXHAUSTION

Proper conditioning and training, coupled with judicious pacing by the rider, are the prime safeguards against EHS. A skilled horse person can bring a mediocre horse through a ride in top condition. The reverse is not necessarily true.

Horses in training must be on a well balanced ration with adequate caloric intake to prevent weight loss. Endurance horses tend to be trim but should not be emaciated. All kinds of diets are touted by endurance enthusiasts. No one regimen has proven to be superior to all others.

One word of caution: Do not recommend heavy calcium or other electrolyte supplementation before the ride. The horse's body may habituate to hyperintestinal absorption of the electrolytes and fail to adapt metabolic systems to cope with electrolyte deficiencies.

During the ride it makes sense to supply balanced electrolytes in water offered.

On 50-mile rides, many well-conditioned horses will not drink. No horse can go 100 miles without drinking and all horses should be allowed to drink whenever possible.

CONGRESS PAPER REFERAAT

# VETERINARY PROBLEMS DURING ENDURANCE TRAIL RIDES

MURRAY E. FOWLER*

Medical problems encountered on endurance trail rides can vary from simple lacerations to major metabolic insults or to full-blown crises involving sophisticated equipment to solve the problem, as in the case of a horse that fell off a trail into terrain from which it could not climb out.

Veterinary surgeons should be prepared to deal with many different types of problems. I have chosen to deal with the more important and commonly seen disorders that will occupy the bulk of one's time while on a ride.

While many of the problems are of an accidental nature, some are directly related to ambient temperature and humidity, the speed or pace of the event and, most importantly, the judgement of the rider. Be alert and take preventive steps as indicated.

## HYPERTHERMIA

Serious hyperthermia or heat exhaustion is rarely encountered in endurance horses. However, the veterinary surgeon should be prepared to deal with hyperthermia on excessively hot days. On some rides, I have encountered daytime temperatures in excess of 38°C (100°F) for 6–10 hours and maximum temperatures of 49°C (120°F) in canyon bottoms.

### Predisposing Factors

Physiologic hyperthermia is a common finding in endurance horses. Muscular activity generates heat. Although the horse can cool itself quite admirably by sweating, the pace required of an endurance horse will likely generate more heat than can be dissipated readily, and body temperatures will rise.

High ambient temperatures and high humidity exacerbate the problem of cooling the horse. Dehydration accompanies evaporative cooling. If dehydration becomes severe enough, it will inhibit further cooling and allow progression of hyperthermia.

### Pathophysiology

The production of heat is self evident. Hyperthermia increases metabolic activity and cellular oxygen consumption (10 % for each degree C rise in the human being) (7). In mammals at body temperatures above 41°C (105,5°F), oxygen utilization exceeds the oxygen supplied by normal respiration, initiating hypoxic cellular damage. The brain, liver and kidneys are most likely to manifest such damage.

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### Clinical Signs

Clinical signs of hyperthermia include increased heart and respiratory rates. The horse sweats profusely in the early stages. As the temperature continues to rise, the animal dehydrates, and sweating declines and may cease. As hyperthermia accelerates, the pulse becomes weak and the animal shows signs of restlessness, dullness and incoordination.

Hyperthermia is characterized by peripheral vasodilatation in an attempt to cool the blood. This results in relative hypovolemic shock. Convulsions (cerebral anoxia) and collapse, rapidly followed by death, result if temperatures rise and remain for long above 42–43°C (107,6–109,4°F).

### Therapy

Cool the horse as quickly as possible. Shower with cool water. Give cold water enemas. Rehydration with fluid per os or intravenously is indicated.

## DEHYDRATION

Every horse will dehydrate to some extent on an endurance ride. The degree of dehydration and a horse's ability to compensate and/or rehydrate, will be a determining factor in the successful completion of the ride. The degree of dehydration will be directly related to the amount of fluid lost through evaporative cooling (sweat) which, in turn, is related to the amount and rate of the work performed and the environmental temperatures and humidity.

Fluid and electrolyte imbalances play a major role in all of the metabolic medical problems that develop during an endurance ride. Horses that are eliminated for medical problems consistently have a higher degree of dehydration than horses that go on to finish.

### Clinical signs

Signs of mild dehydration (3 % body weight loss) are low urine output, dryness of the mouth and some loss of skin elasticity.

Moderate dehydration (5 % body weight loss) is accompanied by marked loss of skin elasticity. The eyes are sunken. Blood pressure may fall as a result of decreased plasma volume. Weakness, fever and weak pulse may be observed.

Marked dehydration (10 % body weight loss) may involve circulatory failure from decreased plasma volume.

Simple dehydration is rarely seen in the endurance horse. Electrolyte alterations accompany the dehydra-

tion and will contribute to the overall medical problems that develop.

### Pathophysiology

Body fluid is lost first from the interstitial fluid compartments. Homeostatic mechanisms function to keep plasma volume constant as long as possible. The fluid loss is primarily in the form of sweat but loss from the respiratory tract is also seen.

The fluid volume contraction that occurs in the horse is hypotonic. That is, in spite of the dehydration, there is a decrease in the concentration of most serum electrolytes brought about by significant quantities of chloride, sodium and potassium being excreted in the sweat. The decrease in blood volume is a serious stress to the horse. Compensating mechanisms are brought into play to maintain blood volume. The kidney will conserve sodium at the expense of potassium and hydrogen, exacerbating alkalosis.

Thirst is a protective mechanism that is triggered by blood volume contraction and by hypertonicity of the plasma. Recall that the fluid contraction in the endurance horse is hypotonic. This may account for lack of thirst in some severely dehydrated horses with the exhaustion syndrome.

Weight losses of 10–15 kg/hr have been recorded in endurance-trained horses exercising in high environmental temperatures (32°C) (Carlson). A three-hour ride could result in a loss of 45 litres of fluid in a 450 kg horse. A 10 % body weight loss would constitute severe dehydration.

### Therapy

Allow the horse to drink. Offer small amounts of cool but not cold water. If the horse refuses to drink, gastric intubation may be indicated. Fluid is absorbed from the colon; thus, enemas are effective in rehydration.

In acute dehydration stress, intravenous fluids should be given, preferably balanced electrolytes such as Ringer's solution. Remember that a severely dehydrated horse may have a 45 $\ell$ fluid deficit.

## SYNCHRONOUS DIAPHRAGMATIC FLUTTER (SDF)

SDF is a clinical sign often observed in endurance horses while on a ride. It is defined as a spasmodic contraction of the diaphragm synchronous with the heartbeat. It is not life-threatening in and of itself but is indicative of mild to serious metabolic conditions which may be life-threatening. The development of SDF at any point in the ride should be ample reason to prevent the horse from going further.

### Predisposing Factors

SDF may develop at any time after 20 or 30 miles of riding. There is no sex, age or breed predilection. Horses that have been known to develop SDF previously should be watched closely as there is a tendency to repeat; however, a horse may develop the problem on one ride and never again.

### Clinical Signs

The primary sign of SDF is a spasmodic contraction in the flank area. A horse will usually come into a rest stop in a normal manner but when it returns for the recheck, one will note the thumping in the flank. The "thump" is also easily felt by light palpation in the flank area. If one auscultates the heart while holding a hand over the dorso-caudal rib area, it is apparent that the diaphragmatic contraction is synchronous with the heartbeat.

SDF may be the only clinical sign noted or it may be a part of the exhausted horse syndrome.

The degree of thumping may vary from a barely preceptible quiver to a contraction that seems to rock the horse's body and is observable from a distance. The flutter may be continuous or intermittent, especially in degree.

### Pathophysiology

SDF has been described in man, the dog and the horse in a variety of clinical conditions, including trauma associated with pressure on the phrenic nerve, congenital malpositioning of the phrenic nerve, alkalosis from persistent vomiting and in the hypocalcemic state. The mechanism of action seems to be a hypersensitivity of the phrenic nerve to electrical stimuli occurring in the heart. In the horse, the phrenic nerve passes over the pericardium adjacent to the atria.

The synchrony of the diaphragmatic flutter can be demonstrated manually or electronically. For manual demonstration, a hand is placed on the flank of a horse and the other on the marker button of a simultaneously run electrocardiogram. The marker is punched each time the contraction is felt. It will be quickly noted that the contraction is synchronous with the atrial depolarization as the marker appears just after the p-wave.

Why is the phrenic nerve hyperirritable? The precise etiology is unknown; however, there is good evidence that electrolyte imbalances and alterations in acid base balance are involved. SDF has been noted in clinical conditions in horses not associated with endurance riding. These include salmonellosis, laminitis and uterine torsion. Where these horses have been monitored for plasma electrolytes and blood pH, it has been noted that the horses are usually alkalotic and/or hypocalcaemic. The administration of sodium bicarbonate accentuates the flutter and intravenous administration of calcium gluconate ameliorates the sign.

Progressive alkalosis can result in a corresponding decrease in both ionized and total serum calcium. Hypocalcaemia lowers the threshold of nerves to electrical stimulation, thus predisposing the phrenic nerve to stimulation by the electrical activity of the heart.

Endurance horses are also stressed, so plasma cortisols are elevated. Excessive cortisol lowers calcium levels, increases urinary excretion of potassium and stimulates sweat secretion with the loss of both potassium and chloride which exacerbates potassium deficiency and alkolosis.

Hypokalemia is a consistent manifestation in endurance horses and may also be involved in SDF. Although the usual result of hypokalemia is flaccid paralysis, it has been shown experimentally that hyperirritability of long nerves can also occur (Mansman).

It is apparent that SDF appears following a rest at a checkpoint because the degree of hypocalcemia and hypokalemia and alkalosis may be exacerbated by rehydration without concomitant intake of adequate electrolytes.

In summary, hypocalcaemia, hypokalaemia and alkalosis acting in concert or perhaps individually, are the likely causes of SDF in endurance horses.

### Therapy

Most cases of SDF resolve themselves with rest. Administration of intravenous fluids containing balanced electrolytes may be indicated or if the same fluids can be given per os, this is entirely satisfactory. The addition of calcium salts to the electrolyte solution may be valid therapy. *If calcium is given intravenously,* however, the heart should be carefull monitored.

Alkalosis can be temporarily corrected by having the horse breathe into a plastic bag. The obvious therapeutic contra-indication is the administration of sodium bicarbonate solution. Many veterinarians automatically assume that because the horse has been exercising, it has a lactic acid build up and is acidotic. Not so in endurance horses.

## MUSCLE PROBLEMS IN ENDURANCE HORSES

Musculotendon disorders are commonly encountered in endurance horses. Metabolic myopathies vary from simple muscle soreness through the tying-up syndrome to paralytic myoglobinuria. Traumatic myopathies include contusions, muscle ruptures and tendonitis. Myopathies caused by trauma are handled in a standard manner. The metabolic disorders are more difficult to alleviate or require special treatment.

### *Predisposing Factors*

Muscle integrity and function is dependent upon proper mobilization and utilization of energy. Muscles of the highly trained equine athlete are capable of functioning at a sustained high rate for long periods of time. Nonetheless, there is a limit. If that limit is exceeded, myopathy results.

### Clinical Signs

Mild muscle soreness is characterized by alterations in gait that point toward muscle weakness. The gait becomes progressively altered until the horse is reluctant to move at all. The horse is in obvious pain. There is an anxious expression and excessive sweating. Affected muscles are painful to palpation and may be swollen. Skin temperatures over the muscles may be elevated. Muscle spasms may occur but not consistently. Myoglobinuria is observed in moderate to severe cases and should be considered prima facia evidence for stopping the horse from continuation of the ride.

Myopathy may be one manifestation of the exhausted horse syndrome but is certainly not a consistent sign of the syndrome.

Serum enzymes are markedly elevated but this data is not obtainable while on the ride. CPK levels may vary from 1 000–30 000 IU. The SGOT levels will also elevate.

### Pathophysiology

The energy for muscle work comes from three or more sources but only two systems are important in trail horses.

The primary energy source is the aerobic system utilizing glucose, lipids and oxygen yielding carbon dioxide and water.

A second system involves lactic acid production from anaerobic conversion of pyruvate. The anaerobic threshold in the horse is approximately 600 meters/min (23 miles/hr). If the pace is above this threshold, the horse exceeds its ability to provide energy by aerobic means and must convert to anaerobic glycolysis. Anaerobic glycolysis can only be sustained for 5–10 minutes before the muscle becomes fatigued and must convert back to aerobic metabolism or cease functioning.

It is unlikely that endurance horses attain speeds exceeding the anaerobic threshold for any appreciable time during an endurance ride. One must take this into consideration, however, when highly competitive riders are faced with a close finish. The drive to finish the ride first may be fatal to the horse.

The aetiology of exertional myopathy (capture myopathy) has been studied intensely in wild animals by scientists here in South Africa. Acidosis, electrolyte imbalance and circulatory disorders contribute to the capture myopathy syndrome.

Those working with horses have yet to determine the precise etiology of the tying-up syndrome or paralytic myoglobinuria. Some relate it to dietary deficiencies such as selenium or Vitamin E; others claim defects in carbohydrate metabolism.

The basic lesion in the horse is that of an ischaemic necrosis of muscle fibers. The mechanism for the ischaemia is yet to be determined. There are, however, a couple of hypotheses that are consistent with alterations noted in electrolyte patterns of endurance horses.

Potassium deficiency at the muscle level but not necessarily hypokalaemia, is suspected of being the cause of exertional rhabdomyolysis in military recruits subjected to work-heat stress.

During muscular activity, a significant proportion of the blood will be shunted to the muscles to supply the heavy oxygen and energy demands of work. The stimulus for muscle vessel dilatation may be brought about by release of potassium from the muscle cell. A local deficiency of potassium results in a failure of release of potassium, the failure of arteriolar dilatation, leading to inadequate tissue perfusion and possible ischemic necrosis of muscle cells. Endurance horses usually have hypokalaemia. We do not know, however, what the status of potassium is at the tissue level. Since all endurance horses tend to by hypokalaemic and only a few develop exertional myopathy, other factors must play a part in the production of the syndrome.

Lactic acidaemia is not a likely factor in exertional myopathies in the endurance horse because anaerobic metabolism is rarely encountered. All acid base studies conducted to date indicate that endurance horses may be mildly acidotic but more commonly are alkalotic.

One common phenomenon associated with muscles is the spasm cramp, twitch or fasciculation that frequently accompanies the exhaustion syndrome. This is likely related to hypocalcemia or other electrolyte alterations. Horses with these types of muscle problems can be walked out of it, as opposed to rhabdomyophysics in which exercise is detrimental.

### Therapy

Rest is paramount in all cases. The horse should not be allowed to move except by trailer from the place where

the diagnosis is made. A trailer ride should not be attempted until the horse has cooled out, rehydrated and other therapy has been carried out.

A rider may have difficulty differentiating the pain associated with myopathy to that seen in colic. It is disastrous to force the severely myopathic horse to walk as one would do with a suspected colic. Discuss the problem with riders and explain that colic is quite uncommon but myopathy is common. If in doubt, don't exercise.

Horses in inaccessible locations should not be walked out until all possible recovery has taken place. I have treated and worked with a horse for 24 h in the bottom of a canyon before attempting to take her out to a trailer site.

Horses with mild muscle soreness may improve if walked slowly but rest from ride exertion is still the major therapy.

Phenylbutazone in doses of 2–3 g can be given intravenously for pain. Restore the horse's fluid balance either per os or intravenously using balanced electrolytes. The fluids also aid in flushing the kidneys to speed up the excretion of myoglobin. Excessive myoglobin in the kidney can cause renal shutdown and renal failure.

Phenothiazine tranquilizers may act as vasodilators in standard tranquilizing doses and may cause shock. Steroids are given by some clinicians but their use should not be prolonged. Use of vitamin B complex is standard therapy. Although administration of selenium vitamin E preparations may have some preventive value, there is some evidence that their use as therapy may be counter-productive.

## LAMINITIS

Laminitis is a clinical entity seldom seen during an endurance ride but may be a serious sequela to a ride, leading to the necessity of destroying a horse.

### Clinical Signs

The classical signs of acute laminitis appear as painful locomotion and throbbing digital pulsation. The feet are hot and painful to squeezing with a hoof tester. Diagnosis is not difficult.

### Pathophysiology

Laminitis likely has multiple etiologies, the delineation of which are not all pertinent here. Two hypotheses may be involved in laminitis of endurance horses. Trauma to the foot is the most logical cause of inflammation of the foot. Horses should have been trained in terrain similar to that of the ride but occasionally a horse will encounter excessively rocky going that will pound the feet unmercifully, especially if the rider is not wise enough to slow the pace when negotiating such a trail. Leather or synthetic pads are frequently worn under the shoe. I have seen new pads literally chewed off the foot by rocky trails over the course of 50 miles.

One cannot discount the effect of cardiovascular impairment as a contributing factor to laminitis in endurance horses. Certainly dehydration, electrolyte alterations and acid-base balance problems can alter blood flow to the foot.

Gastroenteric upsets in the horse, including carbohydrate overload, could predispose a horse to the development of laminitis during a ride.

### Therapy

Therapy for acute laminitis is quite controversial. The basic lesion is a malperfusion at the capillary level in the foot which is accompanied by ateriovenous shunting. Therapy should then be directed at reestablishing circulation in the feet. A low volar nerve block will inhibit vascular constriction within the foot. Nerve blocks may need to be repeated 2–3 times/d for several days. Proper circulation of blood in the hoof is partially dependent on the pumping action of the foot while walking. It is now thought that mild exercise is an important aid in preventing damage to the laminae. Exercise the horse slowly in soft ground for 10–15 min every hour for 12–24 h, then stop exercise. If the laminitis is subacute or chronic, excercise will not be beneficial and, in fact, may be contraindicated.

Phenylbutazone in doses of 2–4 g orally may be indicated but corticosteroids are contraindicated.

Although soaking in cold water may give temporary relief to feet sore from laminitis, this practice is actually contraindicated in acute laminitis. Warm water would be appropriate as this would tend to dilate the vascular tree and enhance circulation in the foot.

Laminitis cases are likely to be the most persistent and difficult cases to treat on the ride. Unless such horses are given thorough follow-up care after the ride, there is almost certain to be permanent disability.

## COLIC

Colic rarely develops in the fit, highly conditioned endurance horse but is commonly seen in less highly trained horses on shorter rides.

### Predisposing Factors

All the metabolic disorders common to the endurance horse can have an effect on gastrointestinal motility and, hence, development of colic. The excitement of a ride may initiate either spasmodic colic or ileus. The wise rider brings accustomed feeds. The novice may rely on feed provided by ride management and the horse may react negatively to the feed change.

Colics are usually encountered the night before the ride after trailering to the starting point, after a watering stop, if allowed to graze a strange meadow possibly ingesting toxic plants and at the conclusion of the ride.

### Clinical Signs

Although any type of colic may be encountered in an endurance horse, the common syndrome is that of spasmodic colic. The classical signs need not be enumerated here.

### Therapy

Treat as any spasmodic colic.

## PULMONARY OEDEMA

Pulmonary oedema is a rapidly fatal condition that rarely appears in endurance horses but must be considered.

### Clinical Signs

Dyspnoea and possibly frothy nasal exudation are the prominent signs of pulmonary oedema. In the terminal

stages, cyanosis and agonal struggling are associated with anoxia.

### Pathophysiology

Anything that increases pulmonary arterial pressure can predispose an animal to pulmonary oedema. Very high pressures may cause capillary ruptures resulting in bloody froth.

Pulmonary hypertension may be caused by alveolar hypoxia which initiates pulmonary vasoconstriction. Increased capillary blood volume also causes pulmonary hypertension. The mechanism for this phenomenon is left-side myocardial failure.

This hypostatic pressure of the pulmonary capillaries is 9 mm of Hg lower than the oncotic pressure of plasma proteins. Thus, fluids are maintained in the vascular tree. If the oncotic pressure in the vascular system becomes higher, the shift will be towards fluid leaving the capillaries into the interstitial spaces or the alveoli.

### Therapy

No treatment is effective other than positive pressure ventilation with the horses intubated. Mild cases can be rested; but if clinical signs are severe, the horse will die in a matter of minutes.

## SADDLE AND CINCH SORES

Abrasions or ulceration in the saddle bed or cinch area are the result of either point pressure or excessive friction. Great care must be exercised in evaluating "sores" at the preride examination.

### Clinical Signs

Evidence of current or previous "sores" include white hairs in spots over the withers or saddle bed, scars that may or may not be haired over, thickening of the dermis, alopecia with or without swelling, fresh abrasions and increased sensitivity over the withers and back.

Signs that denote currently active saddle sores which may be ground for elimination from the ride include fresh, open wounds, marked sensitivity and fresh, hot, tender swelling.

### Predisposing Factors

Improperly fitted or maintained tack is the primary cause of saddle sores. A saddle tree may fit a horse properly at the beginning of a trimming period but be unsatisfactory after the horse has trimmed down.

### Pathophysiology

Poorly distributed spot pressure is the prime aetiology for saddle sores. Pressure causes a local ischaemia. If this remains for an extended time, the capillary bed may be damaged. When the pressure is released, blood rushes into the blanched site. Extravasation may occur, causing swelling and pain.

At this point, if the lesion is rested and treated as inflamed tissue, complete healing can take place. If, however, the saddle is reapplied, there is now a lump under the saddle that is subject to frictional abrasion. The abrasion can extend through the dermis resulting in severe ulceration. Remember that sensitivity over the back also may be caused by muscle soreness. Friction can cause saddle sores but less commonly than pressure. Cinch sores are usually caused by friction, leading to blister formation.

### Therapy

Once a sore has developed, one must rest the horse or change tack so that there is no pressure or friction on the lesion. Use extreme caution in recommending cutting holes in pads as the spot pressure at the ring edge may be as detrimental as the original saddle sore. Obviously, riders must keep tack cleaned and in good repair. If the saddle is removed, cold water poured over the back of the horse may minimize swelling.

### Prevention

Toughening the back of some horses is a major job of the trimmer. Proper pads or blankets must be selected for the individual horse. Some riders never completely remove the saddle from the horse's back while on the ride. When they arrive at a checkpoint, they loosen the girth slowly in intervals of 10–15 min to prevent rapid flow of blood into ischaemic areas. This is an excellent procedure and consistent with the known pathogenesis of the lesion.

# GROUP B STREPTOCOCCUS – COMPARISON OF *STREPTOCOCCUS AGALACTIAE* ISOLATED FROM HUMANS AND COWS IN THE REPUBLIC OF SOUTH AFRICA

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ABSTRACT: Van den Heever L.W.; Erasmus Mariana. **Group B Streptococcus – Comparison of *Streptococcus agalactiae* isolated from humans and cows in the Republic of South Africa.** *Journal of the South African Veterinary Association* (1980) **51** No. 2 93-100 (En) Division Veterinary Public Health, Department Pathology, Faculty of Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

The serological and biochemical characteristics of 100 strains of *Streptococcus agalactiae* isolated from quarter milk of dairy cows and of 107 strains cultured from various sites of human patients, were determined and compared.

All the isolates were CAMP-phenomenon and Na-hippurate positive, aesculin negative, fell into Lancefield's Group B and could be placed into one of the 6 recognised serotypes. No human isolates fell into type R but for the rest all the other types were represented in the series of bovine and human cultures. In order of frequency, the human isolates were of type III, II, Ib. X and Ia and the bovine of type II, X, III, Ia, Ib and R.

Of the human and bovine cultures respectively, 34 and 96 % altered litmus milk, 30 and 100 % were sensitive to bacitracin. 32 and 4 % were pathogenic to mice, 82 and 93 % reduced the ultimate pH of glucose broth to 4,2–4,8, 36 and 100 % fermented lactose, 93 and 99 % salicin and 94 and 79 % trehalose. Concerning the combination of lactose/salicin fermentation. 35 and 95 % of bovine and human isolates were +/+, 0 and 5 % were +/−, 59 and 0 % were −/+ and 7 and 0 % were −/−.

Data are summarised in 5 tables and discussed against the background of reports from other countries.

It appears that a proportion of the human infections concern organisms likely to have been derived from bovine sources, either directly or indirectly. Definite classification of South African GBS into either human or animal strains does not seem possible. It is concluded that it would be more correct to refer to the *source* of an isolate instead of inferring that because the organism was primarily cultured from, say human resources, it is necessarily a human *'strain'*.

## INTRODUCTION

*Streptococcus agalactiae* Lehmann and Neuman 1896 is the only species of the genus which can be placed in Lancefield's serological group B by virtue of possession of C-substance determinants[5]. It is a well known and common cause of forms of bovine mastitis which vary from subclinical or latent infections to the acute[11]. World wide reports indicate that 23–85 % of market milk and from 0,4 < 6,9 % of quarter milk samples yield *S. agalactiae* on culture[13]. In South Africa, streptococci, mainly *S. agalactiae,* are held responsible for more than 20 % of cases of mastitis[11] while the isolation of mastitis streptococci from 9,5 % of single can market milk samples and the bulked milk from 11,3 % of producers supplying Pretoria indicates the prevalence of endogenous contamination of our raw milk supplies[30].

Livoni cites Plummer as the first (in 1934) to record with certainty the isolation of a Group B streptococcus (GBS) from man[19]. Since then GBS have been increasingly isolated and recognised as common commensals of the human throat, large intestine and urogenital tract[2 3 6 7 9 10 12–15 18–20 26 32 33]. Some 3–25 % of all streptococci isolated from man are *S. agalactiae*[2 3 13]. Human infection does not, however, necessarily result in clinical disease. GBS are considered to be opportunist pathogens capable, on occasion, of causing a wide range of conditions which include pneumonia, empyema, puerperal fever, multiple abscesses, peritonitis, urinary tract infections, arthritis, meningitis, ulcerative endocarditis, septicaemia associated with thromboembolism and ischaemic cutaneous necrosis in female diabetics, wound sepsis in adults and especially in women and fatal infantile septicaemia and meningitis[2–6 10 13 14 18 19 26 32]. Amongst infections with streptococci of Groups A, B, D, H and N–G, those of Group B are reported to lead to the highest mortality rate[18].

The presence of GBS in the human female genital tract is associated with infection of neonates at parturition with serious illness supervening on predisposing debilitating factors[3]. Jacobs et al. recently recorded details of 16 cases of mostly (62,5 %) fatal neonatal and infantile disease in Johannesburg hospitals which were caused by GBS[14].

In reviewing the situation in 1965, Livoni regarded GBS in man and the cow to be of greater significance and distribution than generally assumed[19 20]. Interest in a possible relationship between bovine and human infections has led to investigations in various countries[3 7 8 14 15 17–23 26 27]. The consensus of opinion appears to be that although *S. agalactiae* isolated from human and bovine sources are morphologically and culturally identical and both belong to Lancefield's Group B, they possess different biochemical characteristics, murine pathogenicity and serological type characteristics. There exists, however, a considerable degree of overlapping of these properties in strains isolated from animals and man[3]. Based on the results of biochemical reactions and serotyping it has been considered that human and bovine strains are separate bacteriological and epidemiological entities[3], regular human infection being independent of any bovine source of infection and vice versa[6 8]. It is, however, conceded that transfer of GBS between man and the cow may occur on occasion[3 8]. On the other hand, a report that the rate of detection of *S. agalactiae* in consumers of raw milk is 40 times as high as in those who use pasteurised suggest a definite interrelationship between bovine and human GBS infections[29].

Butter & De Moor have emphasized that in the Netherlands, definite classification of GBS into bovine and human strains can be based upon haemolytic properties, lactose and salicin fermentation, sensitivity to bacitracin and serotyping[3]. Pomales-Lebrón et al., on the other hand, examined a series of isolates and concluded that in the majority the difference in biological properties between bovine and human isolates was no greater than between individual strains from the same source[25]. They considered the existence of a close relationship between bovine and human strains of GBS[25].

There are a few reports of fish-pathogenic strains of GBS[3 32] and GBS have also been isolated from pigs, dogs, rabbits and lizards[3].



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In view of the prevalence of GBS in milk and dairy herds, the relatively large proportion of milk which is still consumed without any prior heating process, the increasing frequency of isolation of and interest in GBS from human sources and the uncertainty regarding the relationship between bovine mastitis and human GBS infection, the following study was untertaken to examine and compare *S. agalactiae* isolated from man and cows in various parts of the Republic of South Africa.

## MATERIALS AND METHODS

One hundred and seven isolates of presumed GBS from human sources were obtained from laboratories in Rondebosch (41), Cape Town (33), Johannesburg (28), Durban (3) and Pretoria (2) (Table 1). One hundred isolates of presumed GBS were cultured from quarter milk samples drawn aseptically from dairy cows maintained in the following districts of the Republic: Cape Town (35), Pretoria (17), Vereeniging (9), Springs (8), Delmas (8), Olifantsfontein (5), Pietermaritzburg (14), Volksrust (2), Bethlehem (1) and Uitenhage (1). All isolates were examined for possession of C-polysaccharide antigens of Lancefield's Group B by testing autoclaved cell extracts, prepared according to the Rantz and Randall method as modified by the Streptococcus Centre at Kiel, against Wellcome streptococcal grouping serum* in the microcapillary precipitation test. Tests were observed for 15 minutes before being discarded.

Table 1: 107 HUMAN ISOLATES OF GROUP B STREPTOCOCCI: FREQUENCY DISTRIBUTION RELATIVE TO AGE, SEX AND SITE/SOURCE

| Age | No. | Sex | No. | Site/Source | No. |
|---|---|---|---|---|---|
| < 4 weeks | 11 | Male | 35 | CSF | 2 |
| 1–12 months | 13 | Female | 37 | Cervix | 7 |
| 1–10 years | 12 | Unknown | 35 | Urethra | 3 |
| 11–20 years | 7 | | | Vagina | 14 |
| 21–40 years | 15 | | | Prostate | 2 |
| > 40 years | 20 | | | Urine | 4 |
| Unknown | 29 | | | Blood | 6 |
| | | | | Nose | 6 |
| | | | | Throat | 32 |
| | | | | Sputum | 8 |
| | | | | Gastric Juice | 1 |
| | | | | Ear | 2 |
| | | | | Abdomen + Umbilicus | 5 |
| | | | | Perineum | 2 |
| | | | | Tissue wounds | 10 |
| | | | | Unknown | 2 |
| | | | | Subphrenic absces | 1 |

Confirmed GBS were initially tested for the CAMP reaction on Tryptone Beef Blood Agar (TBBA) plates and for aesculin hydrolysis on TBBA plates containing 0,1 % aesculin, the latter being read under U–V light of a wave length of 366 nm. Subsequently each of the isolates were examined as follows:

The ability to hydrolyse Na-hippurate was determined by culture of isolates in 1 % Na-hippurate bovine heart infusion broth at 37°C for 3 d before adding $FeCl_2$ in acidified aqueous solution according to the modified Ayers & Rupp[1] method as described by Facklam et al[9].

*Wellcome Reagents Ltd. Wellcome Research Labs, Beckenham, England.

Two litmus milk tubes were each inoculated with a single drop of a 24 h old culture of each isolate in Todd-Hewitt (T–H) broth and incubated for 3 d at 37°C and 45°C respectively before being examined for development of acidity and clotting.

Fibrinolytic ability was established according to the method described by Tillet and Garner[28]. The mouse pathogenicity of isolates was determined according to the method of Pomales-Lebrón et al.[25].

Hydrolysis of aesculin and growth in bile agar was established by inoculation of plates of nutrient agar containing 0,1 % aesculin and 10 % bile extract with a loopful of an overnight T–H broth culture. Hydrolysis of aesculin was confirmed by inoculation of 0,1 % aesculin-peptone water to which a 10 % aqueous solution of $FeCl_2$ was added after incubation at 37°C for 24 h. Absence of a blackish-brown discolouration was taken as proof of failure.

Bacitracin sensitivity was established by placing discs containing 0,5 u of bacitracin on TBBA plates streaked with an overnight T–H broth culture and examining the plates for growth inhibition zones after 24 h incubation at 37°C. Haemolytic properties were established after culture for 24 h at 37°C on TBBA plates.

The ability to ferment glucose, sucrose, trehalose, lactose, mannitol, sorbitol and salicin was tested by inoculating one drop of a 24 h old T–H broth culture into semi-solid cystein-tripticase agar (CTA) containing one of these sugars and examining for acid production after a 3 d incubation period at 37°C. The ultimate or final pH in semi-solid glucose CTA medium was established after incubation for 3 d at 37°C.

All isolates giving negative biochemical reactions were re-examined by the test concerned before negative results were finally recorded. Serotyping of Group B streptococci (GBS) was undertaken by microcapillary precipitation testing of proved monospecific type sera against heated acid extracts of isolate cells prepared according to the Jelinkova method used by the Institut für Hygiene der Bundesanstalt für Milchforschung, Kiel. (G Hahn, Personal Communication, 1978)

The following reference strains for the production of typing sera were obtained from Dr G Hahn: Ia – "090"/B/59; Ib – "H36"B/B59/59; II – "18R521"/B60/59; III – "6313"/63/3/36; R – "Compton"/B25/60 and X – "Compton"/B24/60.

A non-typable strain 630/NT was employed as a further control of our typing sera, while S107 Maxted was used for additional absorption in cases where Ia-antiserum contained antibodies to group B-antigen.

Type specific sera were prepared by Jelinkova's method as recommended by Dr G Hahn (personal communication, 1978) as follows: after incubation for 24 h at 37°C, the T–H broth, inculated with the particular reference strain was centrifuged and the sediment washed 3 times in phosphate-buffer. The suspension was inactivated by heating at 56°C for 1 h. After proof of sterility, the suspension was stored at 4°C for use as vaccine.

Mature rabbits were given 0,5 mℓ of vaccine intravenously and after 1 week, 1,0 mℓ i/v on alternate days during the 2nd, 3rd and 5th week. The antibody titre of a small quantity of serum was determined before exsanguination by cardiac puncture of rabbits producing sera of adequate titre.

For production of known type-specific antisera the absorption strain was pre-incubated at 37°C for 6 h in

T-H broth before subculture into one ℓ of T–H broth and incubation at 37°C for 24–48 h. The broth was inactivated in a waterbath at 60°C for 1 h; after proof of sterility the culture was centrifuged and the sediment washed twice in sterile physiological saline solution before preservation with merthiolate 1:5000. The sediment was then suspended in 30 mℓ of normal saline and sterility was again established before storage at 4°C. For absorption, 1 volume of sediment suspension was mixed with 2–3 volumes of antiserum and held for 4 h at 37°C whilst being gently shaken from time to time. The cellular material was removed by centrifugation and the supernatant serum tested for monospecificity towards an extract of the homologous reference type strain. Absorption proceded as in the following scheme:

| Antiserum strain | Absorption strain |
|---|---|
| Ia | Ib |
| Ib | Ia |
| II | Ib + III |
| III | Ib + R |
| R | III + X |
| X | R |

For serotyping each isolate was incubated in T–H broth at 37°C for 18 h, centrifuged and the sediment washed in normal saline before addition of 0,35 mℓ of N/5 HCl and heating for 2 h in a waterbath at 50–52°C. The suspension was adjusted to pH 7,3 before sedimentation was effected by centrifugation and the clear supernatant bacterial extract used as antigen.

## RESULTS

The results of tests to establish the biochemical properties of the 100 bovine and 107 human strains of GBS are summarised in Table 2.

Table 2: SUMMARY OF BIOCHEMICAL CHARACTERISTICS OF 100 BOVINE AND 107 HUMAN ISOLATES OF GROUP B STREPTOCOCCI *(S. AGALACTIAE)* FROM VARIOUS REGIONS OF THE RSA

| | Number Positive Bovine (=%) | Human (%) | Characteristic of GBS & author ref. no. |
|---|---|---|---|
| Haemolysis: | | | α, β or γ[5 13] |
| – α | 32 | 52 (48,6) | |
| – β | 2 | 28 (26) | |
| – γ | 66 | 26 (24) | |
| CAMP phenom. | 100 | 107 (100) | mostly[5]; all[13] |
| Hydrolysis of: | | | |
| – Aesculin | 0 | 0 (0) | Neg.[13] |
| – Na-hippurate | 99 | 107 (100) | Pos.[5 13] |
| Growth in: | | | |
| – Aesc 10 % bile | 100 | 106 (99) | Pos.[13] |
| – Litmusmilk at 37°C | *96 | *36 (33,7) | Red/Clot[13] |
| – Litmusmilk at 45°C | 0 | 0 | |
| Sens. to Bacitracin | 100 | 32 (29,9) | |
| Fibrinolytic: + | 20 | 10 (9,3) | Neg.[5 13] |
| Fibrinolytic: ± | 23 | 15 | |
| Murine pathogenic | 4 | 34 (31,8) | Low; H›B.[25] |
| Final pH 4,2–4,8 | 93 | 88 (82,2) | Pos.[5] |
| Fermentation of: | | | |
| Lactose | 100 | 38 (35,5) | Most B. pos.[5]; ±[13] |
| Salicin | 94 | 99 (92,5) | Pos.[5 13] |
| Saccharose | 100 | 107 (100) | Pos.[13] |
| Trehalose | 79 | 100 (93,5) | Pos.[5 13] |
| Mannitol | 3 | 0 | Neg.[5 13] |
| Sorbitol | 3 | 0 | Neg.[5 13] |

*Acid and clot or clot only.

The frequency distribution of the 6 GBS serotypes isolated from human and bovine material in the RSA and regions is summarized in Table 3.

Table 3: FREQUENCY DISTRIBUTION OF SEROTYPES OF GROUP B STREPTOCOCCI FROM BOVINE AND HUMAN SOURCES IN REGIONS OF THE RSA

| Serotype | W. Cape | | Natal | | O.F.S. | | Transvaal | | Total RSA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bov. | Hum. | Bov. | Hum. | Bov. | HUm. | Bov. | Hum. | Bov. | Human (%) |
| Ia | 8 | 4 | 6 | 0 | 0 | 0 | 1 | 1 | 15 | 5 (4,7) |
| Ib | 1 | 14 | 0 | 0 | 0 | 0 | 3 | 4 | 4 | 18 (16,8) |
| II | 11 | 24 | 3 | 2 | 3 | 0 | 23 | 6 | 40 | 32 (29,9) |
| III | 8 | 23 | 0 | 0 | 0 | 0 | 9 | 19 | 17 | 42 (39,3) |
| R | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 |
| X | 8 | 9 | 5 | 1 | 0 | 0 | 9 | 0 | 22 | 10 (9,4) |
| Totals | 36 | 74 | 14 | 3 | 3 | 0 | 47 | 30 | 100 | 107 |

Table 4: FREQUENCY DISTRIBUTION OF PERCENTAGE OF HUMAN (H) AND BOVINE (B) GBS IN VARIOUS COUNTRIES WHICH FALL INTO LACTOSE/SALICIN FERMENTATION CATEGORIES

| Ferm. Patt. | USA[7] | Neth.[3] | Germ.[21] | Denm.[16] | RSA |
|---|---|---|---|---|---|
| Lact/Sal. | B–H | B–H | B–H | B–H | B–H |
| +/+ | 36–68 | 64–23 | 88–93 | 97–X | 95–34,5 |
| +/– | 32–0 | 36–0 | 8,4–6,6 | 2,4–X | 5–0 |
| –/+ | 0–58 | 0–77 | 1,6–0 | 1,1–X | 0–58,8 |
| –/– | 0–0 | 0–0 | 1,6–0 | 0–X | 0–7 |

X = Not Reported

Table 5: CLASSIFICATION OF 100 BOVINE AND 107 HUMAN ISOLATES OF GROUP B STREPTOCOCCI INTO FERMENTATION (F) TYPES ACCORDING TO JENSEN[16]

| F | Bovine | Human |
|---|---|---|
| I | 74 | 33 |
| II | 1 | 1 |
| III | 18 | 3 |
| IV | 5 | 0 |
| V | 1 | 0 |
| VI | 0 | 58 |
| Untypable | 2 | 12 |

The lactose/salicin fermentation pattern, expressed as percentage frequency distribution in our 100 bovine and 107 human isolates, is provided in Table 4 in which comparative data from other countries is also furnished.

Table 5 indicates the situation when some of the biochemical properties are grouped according to Jensens fermentation (F) types[16].

## DISCUSSION

The accurate identification of the organism is obviously important in a study of this nature. Despite the statement that some strains of *S. uberis* may react with Group B antiserum, serogrouping into Lancefield's classification is considered definite[5 13].

### Lancefield's Grouping and Serotyping

The isolates studied in this investigation were mostly classified by the laboratories of origin as presumably Group B or *S. agalactiae* on the basis of one or more screening tests such as haemolytic properties, sensitivity to bacitracin, the CAMP reaction and aesculin hydrolysis tests. Remarkably, we found all except one of these cultures to fall into Lancefield's group B.

All of these proven GBS isolates fell into one or other of the 6 recognised serotypes when tested against our own type – antisera. The absence of untypable isolates is a fortunate situation not commonly encountered. Typing of GBS has been suggested as part of a system for differentiating between bovine and human sources of the organism[3 8]. In the Netherlands and Britain type Ib is considered essentially a human strain[3 23]. This type has, however, been recorded amongst bovine strains in the USA[3] and in Norway (6 %)[12]. Reference to Table 3 shows that 4 % of our bovine strains were of type Ib whereas 4 times as many of the human strains fell into this serotype. Clearly our situation resembles that in the USA, Norway and Germany. Although a strain of this serotype is more likely to be of human origin this is not necessarily so.

Further reference to Table 3 indicates that amongs South African GBS of human origin, serotypes III, II, Ib and X predominate in decreasing order of frequency. In Norway the situation is correspondingly reported as reading II and R, Ib and III[12]. In Germany the situation would read Ia, Ib, III and R[15]. In Norway, none of the human strains fell into serotype X[12]; 9,4 % of our human GBS were of this serotype. In Norway, 20 % of human strains were of serotype R[12] and this type also occurs rather frequently in German human isolates[15]. Not a single of our human strains were of serotype R.

Considerable variation therefore occurs from country to country in regard to the serotypes into which human strains fall. This variation is likely to be of lesser magnitude if larger numbers of isolates were to be examined to provide statistically more reliable data. The same applies to bovine strains. It certainly indicates that categoric classification of strains of GBS of unknown origin on the basis of serotyping alone is not reliable.

The data in Table 3 clearly reflects the situation in South Africa when rearranged in percentage frequency of occurrence of the serotypes amongst bovine and human strains in decreasing order:

| Human | Bovine |
|---|---|
| III (39,3) | II (40) |
| II (29,9) | X (22) |
| Ib (16,8) | III (17) |
| X (9,4) | Ia (15) |
| Ia (4,7) | Ib (4) |
| R (0) | R (2) |

Reference to Table 3 indicates that it is not possible to state that any particular GBS is necessarily of human or bovine origin on the basis of type only.

### Biochemical Properties

The ability to hydrolyse sodium hippurate is considered a firm basis of identification of *S. agalactiae*[2 5 6 13] but there are authors who state that this property is variable although usually present[16 21]. Some authors provide details of the prevalence of strains which do not possess this ability[1 3 7 9 17] whilst others have indicated how human and bovine strains differ in this regard without being in agreement regarding identification of the origin of the isolates. Of the few icthyopathogenic strains examined, some hydrolysed hippurate[31] and others not[3]. The situation is much the same regarding other identifying characteristics sometimes considered specific. Thus whereas the organism should not grow at 45°C[5], there are reports of strains that do so[31]. Growth in 40 % bile is said to be characteristic of *S. agalactiae*[5 6] but some GBS will not do so[30].

Whereas such variance may be expected of an organism which has adapted to a variety of hosts such as the cow[5], man[5], fish[3 30], pigs[3], doves[3], rabbits[3] and lizards[3], the possible role of the human factor as well as variations in the cultural media and techniques cannot be disregarded.

### Bacitracin Sensitivity

Sensitivity to bacitracin is an important characteristic of streptococci and the test is widely used for provisional classification of β-haemolytic isolates from human sources, Group A streptococci being sensitive to bacitracin while most Group B (of human origin) are insensitive[2 3]. Sensitive strains were inhibited by concentrations as low as 0,1 $\mu$/m$\ell$ whereas insensitive strains were not inhibited by concentrations as high as 5,0 $\mu$/m$\ell$ in a study of 100 randomly selected strains[3].

In the Netherlands, 87 % of human origin isolates were recorded as insensitive while 99,6 % of the bovine isolates were sensitive in vitro[3]. In the USA all human isolates were insensitive[2 32] whereas all the bovine and one of 2 icthyopathogenic GBS were sensitive[32]. On the other hand, only one of 33 bovine isolates from Curacao were sensitive[3].

In our study we found that 29,9 % of isolates from human tissues were sensitive to bacitracin while all from bovine mammary secretions were sensitive. While it is clearly more than likely that a sensitive GBS is of bovine origin, the method alone does not provide a basis for absolute differentiation between bovine and human isolates. As almost one third of our human isolates were aberrant to what is assumed, i.e. they were in fact sensitive to bacitracin, preliminary classification of β-haemolytic streptococci of human origin into either Group A or B on the basis of bacitracin sensitivity appears to be an unreliable procedure. If, on the other hand, one accepts that bovine strains are more

than likely to be sensitive and that human strains are usually insensitive[3], then our findings must indicate that almost 30 % of human patients in our survey were infected with GBS originally derived from bovine sources.

### Fibrinolysis

Deibel et al. state that *S. agalactiae* does not lyse human fibrin[5]. It was therefore surprising to record and confirm that 20 % and 11 % respectively of our bovine and human GBS (none of which hydrolysed aesculin while all were CAMP-positive and hydrolysed Na-hippurate) brought about completely fibrinolysis and another 23 % and 15 % respectively caused partial lysis. Such fibrinolytic properties are considered to be characteristic of *S. pyogenes* (Group A) and *S. equisimilis* (Group C)[5]. Considering that *S. pyogenes* is β-haemolytic, may on occasion show the CAMP reaction and may or may not hydrolyse aesculin[13], it is perhaps conceivable that some of our human strains that also failed to hydrolyse hippurate may have been incorrectly grouped as B streptococci instead of A. It is highly unlikely that the same could apply to the bovine strains which lysed human fibrin and the reverse holds good for *S. equisimilis*. In all instances the precipitin reactions to Group B antiserum were, however, clear and strong and confirmed.

### Murine Pathogenicity

Regarding the relative pathogenicity of strains of GBS for mice, Hahn et al. have furnished data from 3 authors which indicate that an average of 67,8 % of human isolates and only 8,5 % of bovine strains were pathogenic to mice[13]. Such pathogenicity is, however, also dose related. Thus in the series examined by Pomales-Lebrón et al., none of the mice which received 0,1 mℓ quantities of culture of 50 bovine strains succumbed after intraperitoneal injection, while 4 % died after receiving 0,5 mℓ of culture; conversely 30 % of 20 human strains were lethal to mice on injection of 0,1 mℓ of culture while 65 % died on injection of 0,5 mℓ of culture[25]. In general, our findings are in agreement with those of these authors in that only 4 % of our bovine strains and almost 32 % of our human strains proved to be pathogenic for mice.

### Carbohydrate Fermentation

We were surprised to find that 97 % of our bovine isolates fermented mannitol and sorbitol whereas *S. agalactiae* is stated not to possess this ability[13]. Fermentation of sorbitol is an identifying characteristic of *S. pyogenes animales* C which may or may not ferment mannitol and is β-haemolytic but this organism is CAMP-negative and therefore not easily mistaken for *S. agalactiae*. *S. uberis* (Group E) characteristically ferments mannitol, may ferment sorbitol and produce α-haemolysis and is ± CAMP-positive. It sometimes does not grow at 45°C but it strongly hydrolyses both aesculin and hippurate. We therefore do not think that our isolates could have been *S. uberis*.

Great significance is attached to the ability of GBS to ferment either lactose or salicin or both although reports on strains examined in different countries vary considerably. Whereas Hahn et al.[13] agree that lactose fermentation is variable and that of salicin constant, several authors report on salicin negative strains[3 16 17 21 32]. The consensus of opinion is that whereas bovine strains are usually lactose positive (L+) the situation is more variable in strains isolated from man and other species[3 5 7 12 32]. Reports indicate that most human strains are L−. In Norway, 96 % and 95 % of bovine strains but only 26 % and 27 % of human isolates were L+[12 26]; in the USA the prevalence of L+ strains amongst bovine isolates is varyingly recorded as 100 % and 64,8 % and among human isolates as 0 % and 35,5 %[7 32]; in the Netherlands, all the bovine and only 23 % of human isolates were L+[3]; from Germany it is reported that 100 % of bovine and 25,4 % of human isolates were L+[21]; Hahn et al. summarised available literature reports to come to the conclusion that 85,8 % of bovine and 31,7 % of human GBS isolates ferment lactose[13]. Table 2 shows that all our bovine but only 35,5 % of our human strains fermented lactose and this situation is therefore similar to that obtaining in other countries.

Even greater emphasis is placed upon the combination of lactose and salicin fermenting abilities (L/S). El Ghoroury considered −/+ strains to be of probable human origin as he found no bovine strains in the USA to be in that situation[7]. Butter & De Moor found that all their human isolates were either −/+ or +/+[3]. In viewing the situation in the RSA, reference to Table 4 shows that 58,6 % of human strains were −/+ and 34,5 % were +/+. Our findings also agree with work done in the USA in that we also found no −/+ GBS from bovine sources and no +/− strains from human sources.

In comparing our results relative to the fermentation of lactose and salicin with those reported from other countries (see Table 4) it becomes clear that there is some variation in strain characteristics. In the RSA most bovine but only about one third of the human isolates ferment both lactose and salicin; a similar situation exists in the Netherlands[3] and possibly in Denmark[16] but the reverse has been found in the USA[7] and Germany[21].

Only 7 % of our bovine strains were unable to ferment both lactose and salicin. A few such bovine strains have been recorded from Germany[21] but none from the USA[7], the Netherlands[3] and Denmark[16]. As far as South Africa is concerned it appears that when considering the ability of an isolate to ferment lactose/salicin, the estimated probability is 73 % that it is of bovine origin if it is +/+ and 100 % that it is of human origin if it is −/+. Because of the small numbers involved one could, at most, say that it is unlikely that an isolate is of bovine origin if its fermentation pattern is −/+ or −/−. These estimates of probability are based upon the assumption that the frequency distribution recorded in this survey represents the true probability distribution in the total bovine or human population.

The problem clearly lies with using the L/S system for identifying bovine and human strains when the results of strain culture show a +/+ reading as 34,5 % of human origin cultures may have this pattern. It is, however, far more likely that a +/+ culture is of bovine than of human origin.

Only a very small percentage (5) of our bovine strains could ferment lactose without being able to ferment salicin whereas no human strains fell into this category. This pattern agrees with the situation in the USA[7] and the Netherlands[3] as far as the human strains

are concerned but more than one third of their bovine strains fell into this combination.

None of our bovine isolates could ferment salicin but not lactose and the same situation exists in the USA[7] and the Netherlands[3] while only a very small percentage of bovine strains in Germany[21] and Denmark[16] fell into this category. On the other hand more than half our human isolates were of this combination and this agrees with the situation in the USA[7] and the Netherlands[3] but is in sharp contrast with what has been reported from Germany[21].

The final or ultimate pH of glucose broth brought about by the growth of GBS is critical and should fall in the range of 4,2–4,8[5]. In our study we used semi-solid cystein triplicase agar and found that the lowest pH was reached after 3 d at 37°C. The results of our survey shows that 82,2 % of human and 93 % of the bovine isolates fell within the accepted pH range for *S. agalactiae.*

### Reaction in Litmus Milk

According to Hahn et al.[13] growth of *S. agalactiae* results in reddening (acidification) and clotting of litmus milk at 37°C. Müller[21], however, recorded highly significant differences in the behaviour of GBS of bovine and human origin in this medium: Almost 64 % of human origin strains failed to elicit detectable changes in the appearance of litmus milk whereas only slightly more that 1 % of bovine origin strains is in agreement with those of Müller[21] and from this it is concluded that there is a 94 % estimated probability that a GBS of unknown origin is from a human source if it does not produce detectable changes during growth in litmus milk.

Our own results show certain variations regarding biochemical characteristics but these are not out of line with the variations reported by others in respect of what must be regarded as *S. agalactiae.*

### Relation between Human and Animal Disease/Infection

There is controversy regarding a possible relationship between animal and human disease/infection caused by GBS *(S. agalactiae).* Some authors consider there to be a positive relationship[25] and even refer to GBS disease as a possible zoonosis[13]. Others in the Netherlands[3], the USA[8] and Australia[27] consider that human and animal infections to constitute separate bacteriological and epidemiological entities. Authorities like Eickhoff consider that the regular appearance of serious infections in man is not epidemiologically related to streptococcal mastitis in cows, the major reservoir of human GBS infection being the female genital tract[6]. Nevertheless there is general agreement that under particular circumstances of poor hygiene, occasional transmission from cow to man and vice versa cannot be excluded. Butter & De Moor record 2 fatal cases in the Netherlands involving a 32 year old farmer and an 8 week old child. Both died of sepsis caused by GBS which on the basis of serotype, haemolytic properties, bacitracin sensitivity and lactose/salicin fermentation pattern were considered to be of bovine origin; in both cases direct or indirect contact with cows was possible or likely[3]. The urogenital tract and throat of the child's mother failed to yield any GBS on culture.

The possible transfer of *S. agalactiae* from man to cows is of importance where infections with this organism are thought to have been eliminated from a herd. Livoni et al. report on the fact that on 97 Danish farms where new udder infections in the herd could not be explained by the introduction of new cows, GBS were isolated from the throats of 46,2 % of the 117 persons working on the farms[20]. They were possibly responsible for new udder infections in 53,6 % of the herds concerned. In 3 instances the organisms isolated from the staff were lactose negative (and therefore probably of human strain) and so were the strains isolated from the cows in the corresponding herds[20].

Butter & De Moor record, on the other hand, that examination of throat swabs from 31 milkers and 46 family contacts on 21 farms where GBS had recently been isolated from cows' udders, failed to yield GBS[3].

Authors such as Obiger have shown that cows' udders are susceptible to infection with human strains and that it leads to development of mastitis and the secretion of milk from which the organism could be recovered[22]. He concluded that human strains do not differ from the bovine in their pathogenicity for the udders of cows. The fact that Van den Heever & Giesecke were able to induce mastitis in 7 out of 7 bovine quarters by intrapapillary deposition of small numbers of 6 different South African strains of GBS of human origin confirms Obiger's findings[13]. There is no evidence to show that man is less susceptible to infection with bovine than with human strains of GBS. In a community where GBS still occurs in dairy herds and where raw milk is consumed there appears to be no barrier to transmission from cow to man. Because the percentage of cows that are milked by hand in the Republic is still comparatively high when compared to other western countries and this also applies to the consumption of non-heatprocessed milk, the interspecies transmission of GBS cannot be ruled out. Granted that human pharyngeal and female genital tract carriers of GBS constitute the greatest reservoir of infection for their offspring (and others), such carriers themselves may well originally have derived their GBS from contact with cows or milk. It is, however, also clear that elimination of bovine sources of GBS infection alone cannot at this stage result in any significant reduction in Group B streptococcal disease in man.

Butter & De Moor considered that definite differentiation between human and bovine strains is generally impossible[3]. They were nevertheless of the opinion that in the Netherlands, bovine and human strains of GBS could be unequivocably defined on the basis of haemolytic properties, reactions in lactose and salicin, sensitivity to bacitracin and serological typing. They agree that this statement does not necessarily apply to the situation in other countries and on the basis of our findings we do not believe that such differentiation could be valid in South Africa.

The one obvious deficiency in any system by which it is attempted to classify a particular culture of GBS into either the human or bovine group is that it ignores the real possibility that, for example, such a culture from a human patient may, in fact, be the result of infection derived from bovine sources – or vice versa. Accordingly it would, to our mind, be far more correct to refer to *isolates from human sources* as such instead of *human strains* or *human isolates.* The same would, of course, apply to material of bovine origin.

## SUMMARY OF CONCLUSIONS

1. Apart from type R, which did not occur amongst the human strains examined, all the 6 known serotypes were represented in our series of GBS cultures from human and bovine sources. Only 2 % of the bovine cultures were of type R.
2. In order of frequency the predominant types in the human series were III (39,3 %), II (29,9 %) and Ib (16,8 %); in the bovine series the order was II (40 %), X (22 %) and III (17 %). The presence of type Ib amongst the human GBS series puts the South African situation in line with that reported from the USA and in sharp contrast to that in the Netherlands and Britain.
3. Although there appears to be a greater probability that a certain GBS may be of either human or bovine origin on the basis of the serotype, categoric classification is not possible.
4. Several of our GBS isolates did not posess all the standard biochemical properties of classical *S. agalactiae*. Tests to establish the CAMP-phenomenon, failure to hydrolyze aesculin, hydrolysis of sodium hippurate, growth in 10 % bile and reduction of glucose broth to pH 4,2–4,8 were usually satisfied.
5. As expected, all of our bovine but only about a third of the human strains fermented lactose. Concerning the dual ability to ferment both lactose and salicin, no −/+ strains occurred amongst the bovine strains and no +/− strains amongst the human isolates. We estimate that there is a 73 % probability that an isolate is of bovine origin if it is +/+ and 100 % that it is human if it is −/+.
6. The majority of our human strains and only a single bovine strain brought about changes during growth in litmus milk.
7. Our study revealed that a bacitracin sensitive GBS is most likely to be of bovine origin but because almost one third of human origin cultures were also sensitive it would appear that presumptive classification of human -haemolytic streptococci as GBS on the basis of insensitivity to bacitracin is an unreliable procedure. Similarly, a bacitracin – sensitive GBS cannot categorically be classified as being of bovine origin.
8. GBS isolated from man are 4 times more likely to be pathogenic to mice than is the case with bovine isolates. The method is, however, not applicable for absolute categorisation of cultures.
9. We conclude that even small numbers of South African strains of CBS from human sources are perfectly capable of infecting the bovine udder via the papillary duct to cause clinical mastitis.
10. There is no absolute means of categorically classifying a GBS as a human or bovine strain. Cross transmission between bovine and man is clearly possible. A strain cultured from man may be the result of infection from a bovine source – and vice versa. There does not appear to be any firm basis for referring to a human or bovine "strain" and preference should be given to "isolates of human or bovine origin."
11. Although clinical and subclinical GBS infections are firmly established within the human population there seems to be no room for complacency about the fact that raw milk supplies are quite frequently contaminated with mastitis secretions containing *S. agalactiae*. This is supported by the similarity of the characteristics of GBS isolated from bovine and human sources. Similarly, GBS infections amongst farm workers may constitute a source of infections for the udders of dairy cows and this can complicate efforts to eradicate *S. agalactiae* from a herd.


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# INFORMATION

# INLIGTING

## PLASTIC LOOP MAY PREVENT MASTITIS

Mastitis is the most costly disease of dairy cattle in the United States, approaching R826 million a year.

According to USDA Science and Education Administration animal scientist, Dr Max J. Paape (USDA, BARC-East, Building 173, Room 103, Beltsville, Maryland 20705), a small plastic loop inserted into each teat of a cow's udder may prevent the desease.

The loop to which Dr Paape refers is made from a polyethylene similar to that used in the manufacture of plastic milk containers. The design was developed by California veterinarian William Kortum.

A piece of the polyethylene, in other words the loop, 11,4 cm in length, is inserted into the teat through a catheter. Once inside the milk cistern, the polyethylene resumes the shape of a loop.

The loop stimulates the animal's natural disease-fighting mechanism, causing a mild irritation. In response, the number of leucocytes in the affected area increases, enabling the mastitis-causing bacteria to be destroyed.

Normally, it takes about 24 hours for the leucocytes to build up to a point where they can destroy the invading bacteria. By this time, the bacteria have multiplied to such an extent that the infection may already have become established.

Having the leucocytes already present, in response to the loop, can prevent this build-up. In addition, the loop "programmes" the udder to respond faster in the case of infection.

In tests at the Beltsville Agricultural Research Center (USDA, Beltsville, Maryland), Dr Paape inserted the plastic loops into two quarters of the udders of six cows. (He notes that the loops have remained in place for over a year, thus far). All quarters were then infused with toxins produced by *Escherichia coli*, the bacteria which cause a number of mastitis infections.

Dr Paape found that the teats with loops responded more quickly to the toxin, and the leucocyte count increased rapidly in these quarters. He notes that there were four times as many leucocytes in teats containing the loops, as there were in the controls.

Dr Paape believes that the number of leucocytes in the teats containing the loops should be sufficient to prevent the mastitis bacteria from becoming established. Tests in which bacteria are infused into the teats, are now being conducted.

Further studies showed that the loop did not affect milk yield, "solids-not-fat", per cent protein, or per cent of fat in the milk, according to the researcher. Furthermore, the loop had no apparent effect on the overall milk leucocyte count, says Dr Paape, who notes that the increase in leucocytes was evident only in the first 20 mℓ of milk (which is normally stripped off before milking).

Dr Paape is continuing his mastitis research in France, for a year, conducting tests on dairy cattle. He plans to use loops in two teats of each of 20 cows. These cows will then be infused with mastitis-causing bacteria, and the results studied.

Large-scale tests, under dairy farm conditions, are being conducted by Dr Kortum, the veterinarian who developed the loop. These tests are being carried out in cooperation with the University of California's Veterinary Extension Division at Davis (California 95616).

The tests are needed before the loops can be recommended for mastitis prevention, and made available to dairy producers.

("New Loop in Mastitis Prevention": Agricultural Research, Vol. 28, No. 3, September, 1979, U.S. Department of Agriculture, Washington, D.C. 20250)

# INDUCED PARTURITION IN CATTLE. II. PLASMA OESTROGEN AND PROGESTERONE LEVELS

H.M. TERBLANCHE AND J.M. LABUSCHAGNE

ABSTRACT: Terblanche H.M.; Labuschagne J.M. **Induced parturition in cattle. II. Plasma oestrogen and progesterone levels.** *Journal of the South African Veterinary Association* (1980) **51** No. 2 101-103 (En) Department of Genesiology, Faculty of Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Plasma oestrogen and progesterone levels were determined in cattle at the time of dexamethasone induced parturition. Oestrogen levels were elevated from the day after dexamethasone administration as well as on the day of parturition but declined rapidly to low levels on the day following parturition.

Progesterone levels showed a decrease from the day of dexamethasone administration until parturition with a rapid drop to very low levels on the day following calving. These results are similar to those reported by other workers.

## INTRODUCTION

The mechanisms responsible for the initiation of parturition in sheep are well known owing to the pioneering work of Liggins *et al*[10]. The important role played by the foetus in this mechanism has been emphasized[4 10 11 17 18] and some of these hypotheses can also be applied to the bovine. These include the increased activity of the foetal hypothalamus, pituitary and adrenal shortly before parturition. This leads to increased cortisol production, increased oestrogen and prostaglandin levels and decreased progesterone levels, as a result of which the cervix will gradually relax and dilate, uterine contractions will increase and the foetus will be forced into the birth canal. Practical application of these hypotheses has led to the artificial induction of parturition in late pregnancy in the bovine with corticosteroids in recent years[2 3 9]. At the same time the question can be put as to whether the hormonal pattern found during induced parturition is the same as that found during normal parturition.

The present study was undertaken to compare the oestrogen and progesterone levels at the time of dexamethasone induced parturition to those found at the time of normal parturition.

## MATERIALS AND METHODS

The clinical procedures and results obtained for dexamethasone-induced parturition in 17 Friesland heifers have been reported[16]. Friesland heifers between 24 and 27 months of age were induced with a single intramuscular injection of 39 mg dexamethasone on the 267th day of pregnancy. The animals were observed at 4 hour intervals until voiding of the afterbirth and the time from induction to birth and from birth to voiding of the afterbirth noted. Blood samples were collected from 16 of these animals commencing on the day of, but prior to, dexamethasone treatment, and ending 12–18 hours after parturition. Heparin was used as an anticoagulant and the samples were centrifuged within two hours of collection. Samples were stored at −15°C until assayed for progesterone and oestrogen content.

Plasma oestrogen levels were determined by radioimmunoassay using a commercially available kit for oestrone and oestradiol*. The parameters of reliability of this assay were not determined. Plasma progesterone was determined with a rapid competitive protein-binding assay of proven reliability[14 15].

*Biokit-Biolab $^{sa}_{nv}$, Belgium, Weil Organisation (Pty) Ltd., Johannesburg.

Statistical analyses were performed on a Hewlett-Packard HP-97 calculator with pre-recorded programmes for mean, standard deviation and t-statistics.

## RESULTS

Oestrogen levels were found to be 0,78 ng per mℓ ±0,39 (n = 17) on the day of, but prior to, dexamethasone administration. These levels were elevated within 24 hours to 1,19 ng per mℓ ±0,31 (n = 16) and remained so until and including the day of parturition. The levels dropped rapidly after parturition, reaching values of 0,25 ng per mℓ ±0,13 (n = 14) within 24 hours of calving (Table 1) (Fig. 1).

Progesterone levels (Table 2) (Fig. 1) averaged 6,33 ng per mℓ ±2,11 (n = 17) on the day of, but prior to, dexamethasone administration. The levels dropped within 24 hours to 1,44 ng per mℓ ±0,44 (n = 16) on the day of parturition. Levels of 0,35 ng per mℓ ±0,22 (n = 14) were found within 24 hours after parturition.

The difference in oestrogen and progesterone levels determined on the day before dexamethasone administration and on the day of calving, and the difference in the levels determined on the day of calving and the day after calving were all highly significant ($p<0,001$).

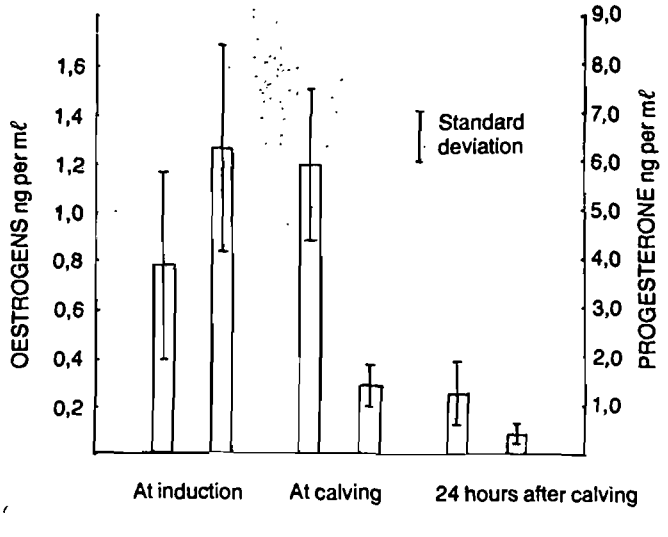


**Fig. 1.** Mean oestrogen and progesterone levels (ng per mℓ) at induction, at calving and 24 hours after calving.



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Table 1: PLASMA OESTROGEN LEVELS AT THE TIME OF INDUCED PARTURITION (NG PER ML)

| Heifer No. | 673 | 674 | 675 | 676 | 825 | 826 | 827 | 349 | 744 | 745 | 746 | 747 | 209 | 210 | 211 | 348 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day of pregnancy: 266 | — | — | — | — | — | — | — | — | — | 0,27* | — | — | — | — | — | — |
| 267 | 1,41* | 0,24* | 0,72* | 0,63* | 0,72* | 0,36* | 0,51* | 0,51* | 0,42* | — | 0,63* | 1,26* | — | 0,90* | — | — |
| 268 | — | — | 1,13° | 1,22° | 1,13° | — | — | — | — | 0,74° | — | — | — | — | 1,21* | — |
| 269 | 1,92° | 0,97° | 0,06 | 0,18 | 0,48 | 0,85° | 1,10° | 0,99° | 0,83° | — | — | 1,53° | — | 1,52° | 1,43° | 1,05* |
| 270 | 0,30 | 0,18 | — | — | — | 0,24 | 0,21 | 0,48 | 0,18 | — | — | 0,18 | — | 0,23 | 0,45 | — |
| 271 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 272 | — | — | — | — | — | — | — | — | — | — | 1,08° | — | — | — | — | — |
| 273 | — | — | — | — | — | — | — | — | — | — | — | — | 0,92* | — | — | — |
| 274 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1,44* |
| 275 | — | — | — | — | — | — | — | — | — | — | — | — | 1,23° | — | — | 1,38° |
| 276 | — | — | — | — | — | — | — | — | — | — | — | — | 0,18 | — | — | 0,18 |

*Dexamethasone administration
°Parturition

Table 2: PLASMA PROGESTERONE LEVELS AT THE TIME OF INDUCED PARTURITION (NG PER ML)

| Heifer No. | 673 | 674 | 675 | 676 | 825 | 826 | 827 | 349 | 744 | 745 | 746 | 747 | 209 | 210 | 211 | 348 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day of pregnancy: 266 | — | — | — | — | — | — | — | — | — | 9,7* | — | — | — | — | — | — |
| 267 | 6,2* | 3,95* | 9,35* | 5,0* | 6,4* | 5,25* | 4,95* | 7,15* | 8,35* | — | 8,25* | 9,7* | — | 2,7* | — | — |
| 268 | — | — | 2,2° | 1,6° | 2,0° | — | — | — | — | 1,95° | — | — | — | — | 4,57* | — |
| 269 | 1,4° | 0,9° | 0,55 | 0,25 | 0,75 | 1,5° | 1,1° | 0,98° | 1,35° | — | — | 2,0° | — | 0,8° | 1,22° | 4,45* |
| 270 | 0,1 | 0,15 | — | — | — | 0,4 | 0,1 | 0,35 | 0,55 | — | — | 0,55 | — | 0,2 | 0,3 | — |
| 271 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 272 | — | — | — | — | — | — | — | — | — | — | 1,64° | — | — | — | — | — |
| 273 | — | — | — | — | — | — | — | — | — | — | — | — | 6,35* | — | — | — |
| 274 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5,34* |
| 275 | — | — | — | — | — | — | — | — | — | — | — | — | 1,45° | — | — | 0,95° |
| 276 | — | — | — | — | — | — | — | — | — | — | — | — | 0,1 | — | — | 0,6 |

*Dexamethasone administration
°Parturition

## DISCUSSION

The results obtained in the present study are in good agreement with those obtained by Edqvist *et al.*[6] in cows and they confirm with those of Liggins *et al.*[10], who have reported increased oestrogen levels in sheep at the time of initiation of parturition owing to the action of the corticosteroids released by the foetal adrenal cortex. The same authors have also reported a decrease in the progesterone levels at this time as was the case in the present study.

It is tempting to postulate that the mechanisms responsible for the initiation of parturition in the ewe and in the cow following artificial induction of parturition are very similar or even identical. Owing to an unfortunate and unforeseen problem, corticosteroids could not be determined in the present assay to further clarify the position in the bovine.

Further evidence of the similarity in the initiating mechanisms between the ewe and cow can be obtained from the results of Fairclough, Hunter & Welch[7] and Hunter, Fairclough, Peterson & Welch[8], who have reported a gradual drop in progesterone levels in late pregnancy in cows with a sharp drop in the last 36 to 48 hours before parturition[7 8]. They also measured increased prostaglandin levels 48 to 72 hours before parturition[7] as well as increased foetal corticosteroids and maternal oestrogens[8] in the last 10 days before parturition.

In contrast, Agthe & Kolm[1] have reported relatively constant levels of oestrogen in the last few days before parturition followed by a rapid drop after parturition to very low levels as was the case in the present study and in that of Edqvist *et al.*[6].

The present results differ to a certain degree from those of other workers as far as the plasma progesterone concentrations are concerned. In the present study progesterone levels ranging from 0,8 to 2,2 ng per mℓ were found on the day of parturition whereas lower levels have been reported elsewhere[5 12 13 15]. Our findings are, however, in good agreement with the 1 to 3 ng per mℓ reported by Edqvist *et al.*[6] under similar conditions.

The important fact is that a decrease in progesterone levels following dexamethasone administration was demonstrated, a fact which is in agreement with the theories on the initiation of parturition referred to above[7 8 10]. The very high levels of oestrogens found at this time would also tend to counteract the relatively higher levels of progesterone permitting the process of parturition to proceed.

ACKNOWLEDGEMENT

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# STUDIES ON FELINE BABESIOSIS
## 1. HISTORICAL REVIEW

G.J. FUTTER AND P.C. BELONJE*

ABSTRACT: Futter G.J.; Belonje P.C. **Studies on feline babesiosis 1. Historical review.** *Journal of the South African Veterinary Association* (1980) **50** No. 2 105-106 (En) 22 Blue Route, Tokai Road, Retreat 7945, Rep. of South Africa.

A review is given of feline babesiosis in which the lack of clinical and pathological data is evident. No consistently reliable chemotherapy appears to be available.

Babesiosis in cats has not received much attention. In 1904 Lingard & Jennings were the first to report on babesiosis occurring in wild and tame felidae but they neither described nor illustrated the parasites causing the disease[15]. In 1929 Davis was the first to describe babesiosis in Felidae[4]. This parasite, which he named *Babesia felis,* was found in a Sudanese wild cat *(Felis ocreata)*. The parasite was artificially transmitted to domestic cats from which he made the following description:

"A small circular body with faint blue-staining cytoplasm and dark red-staining chromatin. The disposition of the chromatin is usually peripheral; in some it is in the form of a small granule, in others it is dispersed around a varying proportion of the periphery of the parasite. Many of these are vacuolated in the centre, in which case they appear ring-shaped and resemble young malaria parasites. Departures from the circular shape are common. Many of the irregular forms are doubtless produced by the act of making the blood-film, but others appear to result from amoeboid movement of the parasite. Evidence of sub-division in the shape of four daughter individuals disposed in a cruciform manner, is occasionally encountered. In some of these, chromatin alone is visible; in others, faint traces of blue-staining cytoplasm can be made out. The daughter individuals are pear-shaped. In some groups the individuals appear to be attached to each other at the centre of the cross; in others they are disjoined. Erythrocytes are also seen containing four young parasites larger than those just described, in which the blue-staining cytoplasm is quite distinct. Forms of the adult parasite are occasionally met with, the nuclear material of which is greatly increased at the expense of the cytoplasm. Many of these, appearing to consist mainly if not entirely of chromatin, tend to be X- or H-shaped. It would seem probable that such formations are preliminary stages of subdivision. During a period of heavy infection, multiple infected red blood corpuscles are often seen; two or more parasites, each representing a different stage of development may inhabit one red cell . . . A number of parasites, measured by means of a micro-projection apparatus, were found to vary from less than 1 $\mu$ up to 2,25 $\mu$ in diameter, the majority being about 1,25 $\mu$."

Although he found that domestic cats were susceptible to the disease, they did not show any signs of illness. The degree of parasitaemia never rose above 1 %. Observations on 5 splenectomised cats showed a dramatic increase in parasitaemia with anaemia being the most consistent finding. Of the 5 cats, however, 2 died of other causes, 2 were euthanised for histopathology and lived 11 months at which stage 3 % parasitaemia was noted.

Wenyon & Hamerton described a small babesia found in the North American Bay lynx *(F. rufa)*. This was a post morten finding at the London Zoo. The parasitaemia was high, cross forms were evident, as well as smaller forms divided into 2 parasites[21].

In 1937 Jackson & Dunning were the first to describe babesiosis in the domestic cat in South Africa[14]. The report concerns a clinical case in the Stellenbosch area. They recorded 7 % parasitaemia, at least 10 % polychromatophilic cells and also the common occurrence of 2 parasites of equal size closely apposed to each other. They considered the parasite to be similar to *B. felis,* but due to its pathogenicity proposed the name *Nuttallia felis* var. *domestica.* In the same journal McNeil published an article on piroplasmosis of the domestic cat[17]. His observations were based on clinical cases in the Cape Town area. He found that anaemia was the characteristic finding and that the cats had a low tolerance to exercise. He also noted that jaundice was only occasionally seen and the colour of the faeces was bright yellow or orange. In his opinion the natural reservoir for the parasite was the wild cat *(F. caffra)* although he was not able to transmit the disease to other cats.

Carpano described *Babesiella felis* in the puma *(F. concolor)*[3]. This was also a small parasite and many erythrocytes apparently contained 4 organisms. Shortt also described a small babesia found in a blood smear of a leopard *(Panthera pardus fusca)*[20]. It has been suggested by Dennig & Brocklesby that all the above parasites should be considered to be *B. felis*[9].

Mangrulkar[16] and Mudalier et al.[18] reported on feline babesiosis occurring in India. Mangrulkar described a small parasite found in a blood smear of a dead Indian cat *(F. domesticus)*[16]. The parasitaemia was very low and no cross forms were noted. On the other hand Mudaliar et al. reported on the findings in an Indian wild cat *(F. catus)*[18]. The parasitaemia was also low and in the absence of these forms and the appearance of paired forms it was decided to name this parasite *Babesia cati.* They were not able to transmit the infection to domestic cats. Dennig & Brocklesby agreed with the classification of the parasite as *B. cati*[9].

Brocklesby et al. isolated a large babesia from a leopard *(B. pardus)* in Kenya[1]. The parasite was sub-inoculated into splenectomised domestic cats which were transferred to Munich for further studies. A small babesia *(B. felis)* was also isolated from a leopard *(P. pardus)* in Kenya, sub-inoculated into splenectomised domestic cats and transferred to Munich. Dennig & Hebel investigated the light and electron-microscopic

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features of these 2 parasites[10]. A large babesia *(B. herpailuri)* was isolated from a jaguarundi *(Herpailurus yaguarundi)* which had originated from South America. The domestic dog was found to be susceptible to *B. herpailuri* whereas it was not susceptible to *B. felis* and *B. pantherae*. The study of these parasites was reported in several publications[5–8 10 11 13]. The investigations concerned morphology, pathogenesis, host specificity and chemotherapy.

Brownlie[2] reported on the use of chlortetracycline° and Dorrington & Du Buy[12] on cephaloridine hydrate (Cepotan, Glaxo-Allenburys) in the treatment of the disease. In the latter 12 clinical cases were presented, of which only one was jaundiced. Robinson described the disease occurring in the Knysna area[19]. Fifteen clinical cases were reported on, the clinical picture being anaemia, some jaundice and temperatures varying from 38,9 to 39,4°C.

All 3 these authors expressed unsatisfactory results in the therapy of the disease[2 12 19]. Brownlie[2] used quinuronium sulphate§, phenamidine isothionate†, trypan blue and arsenicals with very little response. On the other hand he found intravenous euflavine‡ and oral Aureomycin° to be effective. Robinson[19], however, expressed dissatisfaction with the use of Phenamidine and 4,4-diamidino diazoaminobenzene* and found that trypan blue and oxytetracycline°° gave good results although in his opinion these drugs appreared only to suppress the parasites. Dennig[6] found that Berenil and quinuronium sulphate°°°, had very little effect on *B. felis*. However, McNeil[17] reported on the effective treatment of *B. felis* using either trypan blue or Acaprin.

Up to the present, most veterinary practitioners have treated the disease with a mixed bag of babesiocidal drugs, antibiotics and antimalarial preparations with varying results.

To date there has been no investigation into the haematological and patho-physiological changes in *B. felis* in domestic cats. In fact, data gained from dogs suffering from *B. canis* has merely been extrapolated and applied to cats. Common terms such as biliary fever and tick bite fever are used, although affected cats are usually not jaundiced and the vector has not been found.

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°(Aureomycin, Cyanamid)
§(Babesan, ICI)
†(Phenamidine, May Baker.)
‡(Gonacrine, May Baker)
*(Berenil, Hoechst)
°°(Terramycin, Pfizer)
°°°(Acaprin, Bayer)

# EXPERIMENTAL INDUCTION OF BOVINE MASTITIS WITH HUMAN STRAINS OF GROUP B STREPTOCOCCI *(STREPTOCOCCUS AGALACTIAE)*

L.W. VAN DEN HEEVER and W.H. GIESECKE*

ABSTRACT: Van den Heever L.W.; Giesecke W.H. **Experimental induction of bovine mastitis with human strains of Group B streptococci *(Streptococcus agalactiae)*.** *Journal of the South African Veterinary Association* (1980) **51** No. 2 107-109 (En) Division of Veterinary Public Health, Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Intrapapillary deposition of 5–30 CFU of 6 separate human strains of Group B streptococci into 7 lactating quarters of 6 cows resulted in clinical mastitis in all. Identical streptococci were subsequently recovered from 6 of the affected quarters. Details of milk somatic cell counts, bacterial isolations and clinical findings are tabulated. The role of GBS in human medicine and the implications of proof that the bovine udder is susceptible to small numbers of GBS from human sources, are discussed.

## INTRODUCTION

Lancefield's Group B streptococci (GBS), also known as *Streptococcus agalactiae,* are very well known as the cause of various forms of bovine mastitis[4 6]. Despite the advent of antibiotics (to most of which they are susceptible) this organism is still found in the udders and milk of cows in many dairy herds[7 15]. In latter years medical literature has reflected an increasing awareness of the fact that GBS may invade human tissues and at times become an opportunistic pathogen capable of causing a variety of pathological states[1-5 7 10 14 16]. Some of these are serious and may be fatal, particularly in new born infants[2 8].

Despite possession of a common C-determinant which places these streptococci in Group B, there is evidence that human and bovine strains are epidemiologically distinct[2 5]. Such differences are based inter alia upon a classification into one of 6 serotypes, according to their ability to ferment lactose and/or salicin and their sensitivity to bacitracin[2]. Others have furnished evidence that human and bovine infections are in fact related, even though indirectly[13] so that a woman may, for example, become covertly infected by contact with bovine infection and then transfer the organisms to her baby at birth. Isolated reports of apparent direct human infection from bovine sources are also to be found[2]. There is also evidence that human carriers of GBS may be responsible for new udder infections encountered in closed herds previously free of *S. agalactiae*[11]. Bovine mastitis has been artificially induced by introduction of human isolates into the bovine udder quarter[12].

This report deals with the details concerning the intrapapillary deposition into bovine udder quarters of small numbers of a series of individual human GBS and the effect of such infection on the mammary tissues and secretion.

## MATERIALS AND METHODS

Bacteria for infection were obtained from cultures of GBS derived from human sources (and of known serotype, lactose/salicin fermenting ability and bacitracin sensitivity: see Table 1). By subinoculation of a standard quantity of a 24 h old Todd Hewit (TH) broth culture into standard quantities of fresh TH broth on 3 successive days and dilution in sterile buffered normal saline (pH 7,2), fresh suspensions of 24 h old cells containing estimated numbers of colony forming units (CFU)/m$\ell$ were prepared.

Table 1: CHARACTERISTICS AND SOURCE OF HUMAN GBS ISOLATES

| Strain No | Source | Serotype | L/S | Bacitracin sensitivity |
|---|---|---|---|---|
| M12 | Sinus | X | +/+ | – |
| M24 | Nose | Ia | –/+ | – |
| M63 | Prosthetic valve | II | +/+ | – |
| M77 | Throat | II | –/+ | – |
| M89 | Throat | Ib | –/+ | + |
| M101 | CSF | III | –/– | + |

(L/S = Lactose/Salicin fermentation)

One week (Day-7) and again immediately before experimental infection on Day 0, foremilk samples were aseptically drawn from the lactating quarters of 2 grade Jersey and 4 grade Friesland cows. The somatic cell content of the milk was electronically determined by means of a Coulter Counter. The milks were cultured for bacteria by streaking 0,01 m$\ell$ quantities onto tryptone blood agar plates for aerobic incubation at 37°C for 18–24 h prior to examination. The quarters selected for infection were, with one exception, bacteriologically negative on both occasions and the somatic cell counts were below 400 000/m$\ell$ (Table 2).

Quarters were infected in the forenoon of Day 0 after complete milking and again on Day 0,25 (i.e. after the next milking 6 h later). Thereafter foremilk samples were drawn aseptically from each quarter before each milking. The infection proceded as follows:

In the case of Jersey 1, a sterile cotton wool pledget carrying 1 drop of a saline suspension of GBS containing an estimated 5 CFU, was aseptically inserted into the teat canal and left there until the following milking some 6 h later when it was removed. After the quarter had been milked out, the process was repeated once. In the case of Jersey 2 and the Friesland cows, either 1 or 2 m$\ell$ of a suspension of GBS in sterile buffered normal saline (pH 7,2) were injected into the teat canal of an emptied quarter. The quarter was milked out after 6 h and the procedure repeated once. Details of strain and the number of CFU of GBS so introduced are indicated in Table 2. The infected quarters were regularly sampled and examined at morning and afternoon milkings (after 18 h and 6 h intervals respectively). On Day 3 the cows were either treated or slaughtered.

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Table 2: SUMMARY OF DATA OBTAINED BEFORE AND AFTER INTRAPAPILLARY DEPOSITION (ON DAY 0) OF SPECIFIED STRAINS OF GBS OF HUMAN ORIGIN

| Cow (quarter) | Jersey 1 | Jersey 2 | Friesland 1 (LH) | Friesland 2 (RH) | Friesland 3 (RH) | Friesland 4 (RF) | Friesland 4 (LF) |
|---|---|---|---|---|---|---|---|
| GBS Str. No. | M12(5CFU) | M24(10CFU) | M77(10CFU) | M63(10CFU) | M89(30CFU) | M101(10CFU) | M101(10CFU) |
| Day-7 | | | | | | | |
| SCC $\times 10^3/m\ell$ | 400 | 350 | 90,5 | 85,5 | 260,5 | 80,75 | 560,7 |
| Bact. Culture | Neg. | Neg. | Neg. | Neg. | Neg. | Neg. | H/S.a |
| Clinical | Norm. | Norm. | Norm. | Norm. | Norm. | Norm. | Norm. |
| Day 0: | | | | | | | |
| SCC | 200 | 360 | 84,25 | 74,75 | 724,7 | 77,75 | 709,7 |
| Bact. | Neg. | Neg. | Neg. | Neg. | Neg. | Neg. | H/S.a. |
| Clin. | Norm. | Norm. | Norm. | Norm. | Norm. | Norm. | Norm. |
| Day 0,25: | | | | | | | |
| SCC | 350 | 480 | 224,7 | 1515 | 6982 | 1111 | 4926 |
| Bact. | GBS | Neg. | Neg. | H/S.a. | H/S.a. | GBS | H/S.a. |
| Clin. | Norm. | Norm. | $Sl^1_+T^1_+$ | $Sp^1_+D^1_+T^1_+$ | Norm. | $SlF^1_+D^1_+T^1_+$ | $Sp^1_+F^3_+D^1_+T^1_+$ |
| Day 1,0: | | | | | | | |
| SCC | 880 | 640 | 3124 | 14173 | 1010 | 29008 | 1425 |
| Bact. | GBS | GBS | GBS | GBS | Neg. | Neg. | H/S.a. |
| Clin. | $F^2_+D^1_+T^1_+$ | $F^1_+D^1_+T^1_+$ | $Sp^2_+F^3_+D^1_+T^2_+$ | $Sp^3_+D^3_+T^3_+$ | $Sp^1_+T^1_+$ | $Sp^1_+F^3_+D^2_+T^2_+$ | $Sp^1_+F^3_+D^1_+T^1_+$ |
| Day 1,25: | | | | | | | |
| SCC | 7560 | 8650 | 9965 | 8699 | 7389 | 30138 | 1964 |
| Bact. | GBS | GBS | GBS | GBS | Neg. | GBS | GBS |
| Clin. | $Sp^1_+F^2_+D^2_+T^2_+$ | $Sp^1_+F^2_+D^2_+T^2_+$ | $Sp^1_+F^2_+D^1_+T^2_+$ | $Sp^3_+D^3_+T^3_+$ | $Sp^1_+$ | $Sp^1_+F^3_+D^2_+T^2_+$ | $Sp^1_+F^3_+D^1_+T^1_+$ |
| Day 2,0: | | | | | | | |
| SCC | 16532 | 18320 | 23116 | 31823 | 461,7 | 32629 | 4824 |
| Bact. | GBS | GBS | GBS | GBS | Neg. | GBS | GBS–H/S.a. |
| Clin. | $Sp^1_+F^3_+D^2_+T^2_+$ | $Sp^1_+F^3_+D^2_+T^1_+$ | $Sp^1_+F^3_+D^3_+T^3_+Sy$ | $Sp^3_+D^3_+T^3_+Sy$ | Norm. | $Sp^1_+F^3_+D^2_+T^2_+Sy$ | $Sp^1_+F^3_+D^1_+T^1_+$ |

*Key*

Sp = Sero-purulent secretion
F = Flocules in secretion
Sl = Slimy secretion
D = Dolor (Painful)
T = Tumor (Swollen)
Sy = Systemic reaction
GBS = Group B streptococcus
H/S.a. = Haemolytic *S. aureus*
$^1_+, ^2_+, ^3_+$ = Degree of severity

The GBS isolated from quarter milks on Day 2 were serotyped and their ability to ferment lactose and/or salicin as well as bacitracin sensitivity was determined.

## RESULTS

The intrapapillary deposition of GBS resulted in slight to severe inflammatory reactions in the quarters and this was reflected in changes in the nature of the secretion. The results are summarised in Table 2.

The properties of GBS isolated on Day 2 from the quarters were identical to those of the strains which had been introduced into the papillary ducts of the quarters on Day 0. The strains that were used to infect the quarters were therefore recovered from the quarters showing clinical evidence of mastitis.

## DISCUSSION AND CONCLUSIONS

These results shows that under the relevant experimental conditions, normal lactating bovine udder quarters will develop clearly detectable clinical mastitis after intrapapillary introduction on 2 successive post-milking occasions of relatively small numbers of GBS derived from human sources. This confirms previous reports that the cows udder is susceptible to mastitis as a result of GBS infection from human sources.

It is unfortunately not possible to conclude what the outcome of the mastitis would have been if the cows had not been slaughtered or treated. In many natural cases of subacute to acute clinical mastitis, reversion to covert forms of udder disease may take place, the secretion continuing to contain elevated number of somatic cells as well as the causative organisms. Such reversion may be spontaneous or result from therapy.

The potential danger to the human consumer of raw milk from such chronic to subclinical cases of mastitis is evident. In addition, these findings indicate that where handmilking by carriers of GBS takes place or where milking hygiene is not up to standard, infection of the bovine udder with human strains of GBS is feasible. Apart from man the infected bovine udder is presumably the major permanent source of *S. agalactiae*. Eradication of *S. agalactiae* from a closed herd is most desirable but only becomes a practically feasible objective if infection from human carriers can be excluded.

There is evidence to show that the prevalence of GBS infections is considerably higher in communities where raw milk is consumed than in those where milk is pasteurised[7]. Although regular human infection is independent of bovine sources of GBS, the latter may play a role in particular situations[2,5]. Fatal human infections with GBS bearing the characteristics of bovine strains and under conditions when direct or indirect contact with cattle was likely, are on record[2]. There seems no valid reason why GBS could not be transferred from man to cow and even back to man.

Regular monitoring of milk supplies intended for raw consumption for the presence of *S. agalactiae* and steps to prevent such contamination appears to be justified.

## ACKNOWLEDGEMENTS

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## LETTER TO THE EDITOR — AAN DIE REDAKSIE

# HONDSDOLHEID BY KOEDOES

Sedert die tweede helfte van 1977 kom grootskaalse vrektes voor onder koedoes *(Tregalophus strepsiceros)* in Suidwes-Afrika. Die eerste vrektes is in die suidwestelike gedeelte van Okahandja-distrik opgemerk. Later het die vrektes ook in ander distrikte voorgekom. Volgens skatting het sowat 3 000 koedoes die afgelope 2 jaar in Okahandja gevrek.

Aangetaste koedoes is vreesloos, mak en word dikwels op die plaaswerf, in dorpe en tot selfs binne-in geboue op die dorpe aangetref. Simptome soos tenesmus, wikkel van die stert, salivasie, ataksie, inkoördinasie en parese tot paralise in die terminale stadium van die siekte, word dikwels gesien. Die siekte verloop skynbaar vinnig. Vrektes het binne 48 h na waarneming van die eerste simptome voorgekom.

Die oorsaak van die siekte kon nie met nadoodse ondersoeke bepaal word nie maar op grond van die kliniese simptome is hondsdolheid vermoed. Dié vermoede is dan ook deur die Navorsingsinstituut vir Veeartsenykunde, Onderstepoort bevestig by 38 uit 40 breinmonsters afkomstig van koedoes met verdagte simptome.

Hondsdolheid by koedoes het voorheen sporadies voorgekom en was nie as buitengewoon beskou in 'n ensoötiese hondsdolheidgebied nie. Die geweldige toename in die getal gevalle wat tans by koedoes aangetref word, is onrusbarend. Koedoes is 'n belangrike voedselbron in Suidwes-Afrika en die gevaar wat die hantering van moontlikbesmette koedoekarkasse inhou, kan nie onderskat word nie. Koedoes is ook 'n groot aantrekkingskrag vir jagsafaris en toeriste en dien as 'n belangrike bron van inkomste in die vorm van vleis vir uitvoer en jagtrofeë.

Die toename van hondsdolheid by koedoes dui uitsluitlik op 'n andersoortige episoötologie en 'n omvattende ondersoek daarna word tans onderneem. In hierdie stadium word meganiese besmetting van doringbosse wat algemeen deur koedoes gevreet word en 'n gevolglike kontakoordraging as 'n groot waarskynlikheid beskou. Anders as ander wildsoorte asook beeste in die gebied, is koedoes blaarvreters wat in groepe rondbeweeg en dikwels saam aan dieselfde doringtak vreet. Dit verklaar moontlik waarom die siekte in sy huidige vorm tot koedoes beperk is.

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# EFFECT OF DIETARY LASALOCID ON COCCIDIAL OOCYST NUMBERS, FEEDLOT PERFORMANCE AND WOOL GROWTH OF LAMBS

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ABSTRACT: Couvaras S.; Van Niekerk H.P.; Thomas Shan E. **Effect of dietary lasalocid on coccidial oocyst numbers, feedlot performance and wool growth of lambs.** *Journal of the South African Veterinary Association* **51** No. 2 111-113 (En) Department of Zootechnology, Faculty Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort. Rep. of South Africa.

The effectiveness of lasalocid as a coccidiostat in wool producing feedlot lambs was investigated. Significantly lower ($P<0,001$) oocyst numbers were obtained with the lasalocid diet as opposed to the controls receiving no lasalocid. No significant differences were found with regard to wool growth and related properties such as staple length, crimp, fibre thickness, clean yield and clean wool weight. The lasalocid diet improved the efficiency of feed utilization by 6 %.

## INTRODUCTION

Coccidiosis is frequently a problem in feedlot lambs[5] and results in substantial economic losses to the sheep industry[9] of South Africa. Some of the manifestations of coccidiosis in lambs are reduced feed intake, poor weight gains, poor feed utilization, diarrhoea and often death in instances of severe infestation[6].

Recent reports have shown that coccidiosis may be effectively prevented by amprolium[1 7 12] and monensin[3 6 10]. Aside from the reports on the value of monensin and amprolium as a coccidiostat for lambs, there is little reported research on the effect of any particular coccidiostat on lamb performance[4 8] and nothing on the wool growth of lambs or sheep.

Lasalocid†, also referred to in the literature as antibiotic X-537 and Ro 2-2985, and which is marketed as an aid in the prevention of coccidiosis in chickens, was first isolated from soil samples and is described by Berger *et al.*[2].

The objective of this investigation was to test the efficacy of Lasalocid as a feed additive against naturally occurring coccidial infections in weaned wool producing lambs and on the performance of these lambs under feedlot conditions.

## PROCEDURE

Thirty-four fine wooled (18,81 ± 1,08 micron) weaned Letelle type wether lambs with an average age of four months and with a mean shorn weight of 25,53 ± 3,05 kg were divided into two groups with approximately the same body weight (25,00 ± 2,85 and 25,94 ± 3,23 kg respectively) by means of stratified sampling. The two groups were randomly allotted to a ration with or without lasalocid.

The two groups were kept outdoors in sheltered communal pens of approximately 40 $m^2$ where water and feed was supplied *ad lib.* for the duration of the experiment, which lasted 60 days. Water was supplied by an automatic drinking trough in the unsheltered area. The sheltered area of each pen, which was swept clean once a week, consisted of brick walls on three sides, a roof covering and a concrete floor, and made out approximately one-third of the total pen area. The remaining two-thirds of each pen consisted of a hard gravel floor. The animals were weighed regularly at weekly intervals and the feed consumed per group was also recorded.

The ration presented was formulated to supply a calculated 15 % CP and 12 MJ DE/kg, and thus conforming to NRC (1975) requirements for the intensive finishing of growing lambs. To the diet of the experimental animals lasalocid was added at a rate of 90 ppm. The ingredients and composition of the diet together with the chemical analysis (obtained from Agrilab, Pretoria) is presented in Table 1.

In order to accustom the lambs to the finishing ration, the percentage concentrates was increased gradually over the first 10 days of the adaptation period of 14 days. All lambs were dewormed with Multispec* during the first week of the adaptation period and again with Valbazen† during the fifth week of the experiment. Faeces from each lamb was collected weekly and examined for coccidial oocysts. At the onset of the experiment the number of coccidial oocysts per gram of faeces for the experimental and control groups were 3468 ± 2420 and 3062 ± 3266 respectively.

All lambs were carefully shorn with electric clippers at the onset of the experiment and again at the end of

Table 1: COMPOSITION AND ANALYSIS OF THE FINISHING RATION AND ITS CALCULATED* DIGESTIBLE ENERGY CONTENT

| *Item* | Per cent |
|---|---|
| Ingredient: | |
| Coursely ground lucerne meal | 60,0 |
| Yellow maize meal | 30,0 |
| Soyabean oilcake meal | 9,0 |
| Mineral premix† | 1,0 |
| Chemical analysis: | |
| Dry matter | 93,1 |
| Crude protein | 14,4 |
| Crude fibre | 22,5 |
| Crude fat | 3,5 |
| Ash | 7,5 |
| Calcium | 0,9 |
| Phosphorus | 0,4 |
| Copper (ppm) | 12,8 |
| Digestible energy (MJ/kg) | 12,0 |

*Feed composition tables (Van der Merwe, 1977).
†The mineral premix consisted of 50 % finely ground stock salt and 50 per cent Fermafos – 12P (a commercial phosphate-trace element mixture).

*Section Protozoology, Institute Veterinary Research, 0110 Onderstepoort.
†Registered and marketed as Avatec; Roche Products (Pty) Ltd.

*Mebendazole: Ethnor
†Albendazole: Smith Kline


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the experimental period (60 days later). Wool samples of approximately 120 g were taken from the left mid-side of each lamb and sent to the S.A. Fleece Testing Centre at Grootfontein where they were analyzed for fibre thickness, staple length, clean wool yield and crimp. Rate of woolgrowth and all fleece data were corrected to 12 months growth.

Data were analyzed statistically according to the procedures outlined by Snedecor (1956)[13].

## RESULTS AND DISCUSSION

Effects of feeding a ration containing 90 ppm lasalocid over the total experimental period on the feed intake, feed conversion, gain in body weight, clean wool weight gain and oocyst count are given in Table 2, while weekly means for body mass and oocyst counts are shown in Figure 1 and 2 respectively. The means and standard deviation for fibre thickness (micron) and staple length (mm) were 18,55 ± 0,83 and 158,09 ± 15,62 for the experimental group and 19,07 ± 1,26 and 147,08 ± 17,34 for the control group respectively. Feed intake was not analyzed statistically because of the group-feeding system.

The inclusion of lasalocid in the finishing ration of feedlot lambs resulted in significantly lower ($P<0,001$) oocyst numbers in the faeces of lambs receiving the lasalocid diet (Fig. 2).

Although feed intake was not analyzed statistically, the group of lambs receiving the lasalocid diet had a 60 % improved feed conversion rate – due mainly to the reduced feed intake of this group. Several workers have also reported reduced feed intakes when monensin was given as a growth promotant[11,14].

With regard to clean wool weight, fibre thickness and staple length however, no significant differences were found between lambs receiving the lasalocid diet and those receiving a diet containing no lasalocid.

These data indicate that lasalocid has a marked suppressive effect on coccidia under feedlot conditions and will probably act as an effective prophylactic agent against coccidiosis in feedlot lambs. With regard to its effect on the performance of feedlot lambs there is however still more work to be done.

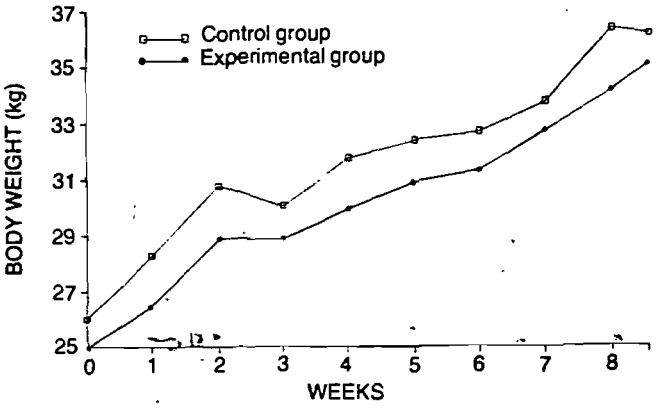


**Fig. 1** Growth curves for the control and experimental groups

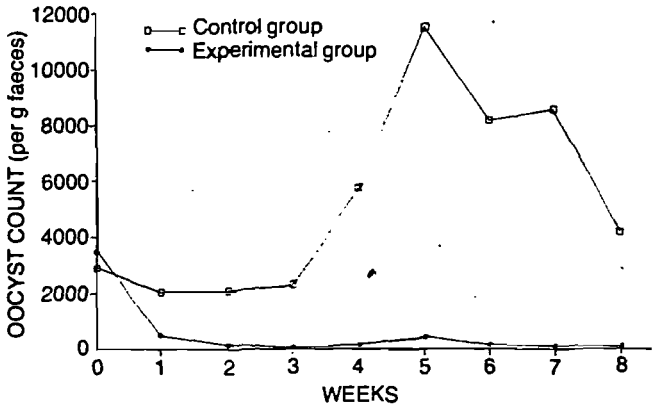


**Fig. 2** Weekly oocyst counts in faeces of lambs


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Table 2: THE EFFECT OF LASALOCID ON THE PERFORMANCE OF WOOL-PRODUCING LAMBS[c]

| Group | Number of animals (n) | Initial body weight (kg ± S.D.) | Dry matter intake[d] (kg/day ± S.D.) | Weight gain gain (g/day ± S.D.) | Feed conversion[c] (kg DM/kg gain) | Clean wool weight[e] (kg ± S.D.) | Oocyst count (per g faeces) |
|---|---|---|---|---|---|---|---|
| Control group | 17 | 25,94 ± 3,23 | 1,50 | 166,67 ± 25,52 | 9,00 | 3,45 ± 0,46 | 4099 ± 3660[a] |
| Experimental group | 17 | 25,00 ± 2,85 | 1,41 | 166,67 ± 46,96 | 8,46 | 3,70 ± 0,68 | 91 ± 115[b] |

[a,b] Means with different superscripts are different ($P<0,001$).
[c] Statistical analysis of clean wool weight and body weight gain indicated no differences ($P>0,05$) due to treatment.
[d] Feed intake and feed conversion not analyzed statistically because of group feeding system.
[e] Corrected to 12 months growth.

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## LETTER TO THE EDITOR — AAN DIE REDAKSIE

# "OSTEODYSTROPHY II"

I am concerned that Stogdale[7], in reviewing forelimb lameness in growing dogs, may have caused confusion by resurrecting the term "Osteodystrophy II". Although Riser[5] used "Osteodystrophy II" to designate a skeletal disorder seen in one dog, he recognised that it might be a variant of hypertrophic osteodystrophy and did not mention it again in a later paper on these conditions[6]. Since then, the term has fallen into disuse and various authors have regarded the changes described as part of the syndrome more commonly known as hypertrophic osteodystrophy[1,4,8] or metaphyseal osteopathy[2]. Several reports have demonstrated clearly the progression of radiographic changes from those described as "Osteodystrophy II" to those typical of hypertrophic osteodystrophy[3,4,8] although not all cases develop obvious soft tissue mineralization and periosteal alterations[2]. These observations suggest that "Osteodystrophy II" represents an early or mild form of hypertrophic osteodystrophy, rather than either a separate entity, or a chronic form of hypertrophic osteodystrophy as intimated by Stogdale[7]. Since Stogdale[7] mentions development in some cases of "Osteodystrophy II" of a "cuff of new bone surrounding the distal metaphysis", a feature specifically excluded in Riser's description of the condition[5], it seems certain that she is in fact describing hypertrophic osteodystrophy. Why then make this artificial distinction to maintain the obscure term "Osteodystrophy II"? Hypertrophic osteodystrophy might not be an ideal name but it is at least widely understood.

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# SAMESTELLING VAN MERINOKUDDES IN DIE REPUBLIEK VAN SUID-AFRIKA

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ABSTRACT: Van der Walt K. **The composition of Merino flocks in the Republic of South Africa.** *Journal of the South African Veterinary Association* (1980) **51** No. 2 115-117 (Afr. En) Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, Onderstepoort 0110. Rep. of South Africa.

The various sex and age components of Merino sheep flocks are analised. Since a similar survey in 1965 the most significant difference is an increase from 40 to 52 % of the mature female component. Various indicators point to an inability of the farmer to influence this slow rate of increase.

The significant role which the prolonged retention of young Merino whethers on the farm plays in this respect is discussed. A higher percentage of rams during the mating period is also suggested.

## INLEIDING

Sonder kennis van die samestelling van die Suid-Afrikaanse skaapkuddes kan sommige probleme moeilik gëdentifiseer word en kan sinvolle beplanning van navorsing en voorligting nie geskied nie.

Daar is derhalwe 'n opname, soortgelyk aan dié van Hofmeyr en Boyazoglu[1], gedoen. Die resultate van die twee opnames, wat met 'n tussenpose van twaalf jaar geskied het, is vergelyk.

## MATERIAAL EN METODES

Gedurende die lamseisoen van 1975/1976 en 1976/1977 is vraelyste onder boere versprei. Onder andere is aan boere spesifieke vrae gestel i.v.m. grootte en ligging van plase, tipe boerdery en die samestelling van die kudde soos weerspieël in die gegewens in tabelle 1 en 2. Die vraelyste is met behulp van opnemers, wat die korrektheid van die inligting beoordeel het, voltooi.

Afsonderlike groepe boere het aan die twee opeenvolgende opnames deelgeneem. Die eerste opname is hoofsaaklik deur takke vn die Nasionale Wolkwekersvereniging (NWKV) behartig terwyl beamptes van Boerekoöperasie Beperk (BKB) goedgunstiglik met die 1976/1977 opname behulpsaam was. Slegs gegewens van boere wat met merinos of merinotipe skape geboer het is verwerk.

## RESULTATE

Die gegewens is ingedeel volgens die sewe landboustreke van die departement Landbou-tegniese Dienste soos aangedui in Fig. 1. Oor die geheel behels hierdie

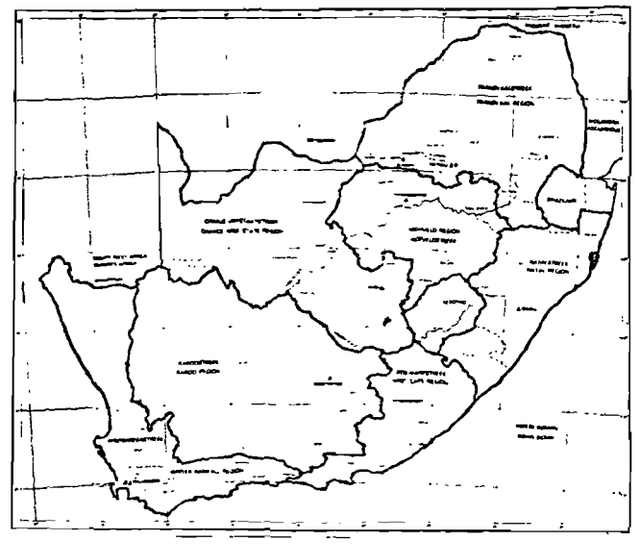

**Fig. 1** Landboustreke van die R.S.A.

landboustreke klimaats- en boerderyomstandighede wat redelik eenvormig is binne elke streek.

Die samestelling van Merinokuddes volgens landboustreke word in Tabel 1 ontleed. Slegs inligting uit volledige voltooide vraelyste en wat as betroubaar beskou is, is verwerk.

Tabel 2 toon die verspreiding van jong en volwasse hamels op plase aan. Die hamelkomponent in die kuddes is afsonderlik ontleed omdat daar beduidenswaardige verskille bestaan in die getalle volwasse hamels wat op plase in die verskillende streke aangetref word.

Table 1: GEMIDDELDE SAMESTELLING VAN MERINOKUDDES VOLGENS LANDBOUSTREKE

| Streke | Plaasgrootte (hektaar) | Kuddegrootte | Weidingsdruk K.V.E./H. | % Ramme | % Volwasse ooie | % Tweetand ooie | % Tweetand hamels | % Volwasse hamels | % Jaarlikse verkope |
|---|---|---|---|---|---|---|---|---|---|
| Karoo | 7164 | 2074 | 3,4 | 2,7 | 58 | 22 | 13 | 7 | 24 |
| Winterreën | 1841 | 1097 | 1,7 | 2,1 | 60 | 20 | 11 | 9 | 27 |
| Oos-Kaap | 2265 | 2102 | 1,07 | 2,1 | 47 | 21 | 15 | 17 | 21 |
| Hoëveld | 1013 | 1010 | 1,0 | 2,3 | 50 | 23 | 12 | 15 | 16 |
| OVS | 2438 | 1810 | 1,3 | 1,7 | 55 | 20 | 15 | 10 | 26 |
| Transvaal | 1663 | 2285 | 0,72 | 2,3 | 43 | 22 | 15 | 20 | 18 |
| RSA | 2723 | 1834 | 1,46 | 2,2 | 52 | 21 | 14 | 13 | 22 |

Tabel 2: GEMIDDELDE VERSPREIDING VAN HAMELS OP PLASE VOLGENS LANDBOUSTREKE

| Streek | % Plase met tweetand hamels | % van kudde | % Plase met volwasse hamels | % van kudde |
|---|---|---|---|---|
| Karoo | 93 | 15 | 43 | 18 |
| Winterreën | 72 | 14 | 45 | 19 |
| Oos-Kaap | 82 | 18 | 55 | 20 |
| Natal | 89 | 17 | 52 | 30 |
| Hoëveld | 83 | 14 | 56 | 24 |
| OVS | 77 | 18 | 30 | 16 |
| Transvaal | 88 | 19 | 76 | 26 |
| RSA | 83 | 16 | 51 | 24 |



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## BESPREKING

### Versameling van inligting

Soos in 1965 is die gegewens vir die huidige opname bekom deur vraelyste. Vanweë relatiewe klein getalle voltooide vraelyste laat hierdie metode heelwat te wense oor maar dit bly die enigste manier om landswyd inligting in te samel.

Daar was werklik twee opnames, deur verskillende instansies, onder afsonderlike groepe boere gedoen. Die resultate was feitlik identies of het nie betekenisvol verskil nie. Hierdie ooreenstemming van resultate verhoog die geloofwaardigheid van die streekproef.

Boere wat die vraelyste voltooi het hou voldoende rekords en toon waarskynlik meer as gewone belangstelling in skaapboerdery as 'n bron van inkomste. Die resultate reflekteer dus toestande op goed bedryfde plase en daar moet aanvaar word dat landswye omstandighede moontlik swakker is as wat hier bespreek word.

### Weidingsdruk

Die Winterreënstreek sluit ekstensiewe weidings aan die droë weskus in sowel as intensiewe graanproduserende plase in die suid-westelike en suidelike distrikte.

Vir die doel van hierdie opname is die gegewens uit die ekstensiewe weigebiede van die weskus weggelaat en resultate verteenwoordig dus omstandighede wat in die suidwestelike graangebied heers.

Gegewens uit die Karoostreek verteenwoordig plase waarop oorwegend maar ook nie uitsluitlik met skape geboer word nie. Die res van die opgawes oor die land kom van plase waar gemengde vee en/of saaiboerdery bedryf word. Die weidingsdruk weerspieël dus plaasoppervlakte en nie slegs natuurlike weiding nie.

Dit skyn asof klein maar belangrike verskille tussen die resultate van Hofmeyr en Boyazoglu en die huidige opname te bespeur is. Die 1965 resultate word deurgaans in die bespreking in hakkies aangegee.

Die gemiddelde plaasgrootte van 2723 h (2247) en kuddegrootte van 1834 (1901) het sedert 1965 die gemiddelde druk van 1,48 skaap per hektaar verminder na 1,18.

Die vermeerdering in plaasgrootte kan waarskynlik toegeskryf word aan die vermindering van die aantal boerderybedrywe en konsolidasie van grond gedurende die afgelope dekade. Die vermindering in die aantal skape per kudde is moontlik teweeggebring deur die daling in getalle wat met die veeverminderingsskema gepaard gegaan het.

### Persentasie ramme

Daar was 'n gemiddelde van 2,2 % (2,1 %) ramme beskikbaar vir ooie wat moontlik gepaar kon word. Die getal varieër van 2,7 % vir die Karoo tot 1,7 % vir die OVS streek. Aangesien alle beskikbare ooie nie noodwendig elke seisoen gepaar word nie sal die werklike ram-ooi verhouding ietwat styg.

Dit word algemeen aanvaar dat onbedrewe jong ramme en jong ooitjies relatief swak reproduksie-resultate lewer. In 1965 het boere gemiddeld 28 % van hulle ramme jaarliks vervang en die huidige opname dui op 'n jaarlikse inname van sowat 20 % jong ooitjies. Die invloed van hierdie komponente op lampersentasie kan dus aansienlik wees en sal die voorgenoemde seisoenale styging in ram-ooi verhouding effektief neutraliseer.

Hoër lampersentasies word van jong ooitjies verkry as hulle in kleiner groepies met 'n hoër persentasie van ouer bedrewe ramme gepaar word. Indien ramgetalle vermeerder word verminder die verskynsel ook van ooie wat periodiek gedurende hulle aanteelouderdom nie lam nie.

Dit word algemeen aanvaar dat 3 % ramme (en hoër in die ekstensiewe weidingsgebiede) in Merinokuddes gebruik behoort te word. Die huidige opname toon 'n landswye tekort van sowat 30 % ramme. As 'n mate van onvrugbaarheid by die reeds bestaande tekort in berekening gebring word is dit waarskynlik dat lae rampersentasies beslis bydra tot onbevredigende lampersentasies.

### Persentasie ooie

In 1965 was die gemiddelde persentasie teelooie in die kuddes sowat 40 % teenoor die huidige 52 %; 'n verbetering van 12 % oor 'n dekade.

Die aantal tweetandooie het in dieselfde tyd effens gedaal van 24 na 21 %. Indien die 52 % teelooie jaarliks elkeen 'n speenlam sou lewer is, as geen verdere verlies voorkom nie, sowat 26 % jong ooitjies vir vervanging beskikbaar. Indien aanvaar word dat ooie hoogstens 4–5 keer gedurende hulle lewe gepaar word, word ten minste 20 % jong ooie per jaar benodig om die aanteelkudde se getalle te handhaaf.

Met ooie wat nie gereeld jaarliks lam nie en verliese vanaf geboorte tot die eerste paringsouderdom is daar 'n besliste vermindering van die moontlike 26 % jong ooie beskikbaar.

Dit wil dus voorkom of die 21 % jong ooie in die huidige opname die maksimum getal beskikbaar is en dat boere oor die algemeen sonder seleksie al die jong ooie jaarliks by die kudde moet voeg.

### Persentasie hamels

Volgens Tabel 2 blyk dit dat die grootste persentasie boere (83 %) nog die meeste van hulle jong hameltjies (16 % van die kudde) vir ten minste die eerste jaar op die plaas hou. Aangesien hierdie syfers gemiddeldes reflekteer is dit nie onrealisties om te aanvaar dat slegs 17 % boere geen hameltjies aangehou het nie en dat die 83 % al hulle jong hameltjies (dieselfde as jong ooitjies, d.w.s. 21 %) gehou het.

'n Verdere 32 % boere hou nie hamels ná die tweetand stadium aan nie, maar 51 % van die Merinoboere hou nog volwasse hamels wat dan gemiddeld 24 % van die kudde uitmaak. Geen inligting is beskikbaar oor hoe gereeld en met watter persentasie jong hamels volwasse hamels vervang nie.

### Jaarlikse verkope

Die jaarlikse verkope wissel beduidenswaardig van 16 % in die Hoëveldstreek tot 27 % vir die Winterreënstreek. Daar skyn 'n mate van korrelasie te wees tussen die getal aanteelooie en jaarlikse verkope. Die Winterreënstreek wat die hoogste aantal skape verkoop het ook gemiddeld die grootste getal volwasse ooie (60 %) terwyl die oooreenstemmende verkope en volwasse ooie vir die Transvaalstreek 18 en 43 % is.

Vir 'n homogene bevolking in die kudde sou die ge-

middelde jaarlikse verkope van 22 % daarop dui dat die gemiddelde verblyf in die kudde van elke skaap sowat vyf jaar is.

Daar is egter twee hoof bronne van verkope, nl. hamels en slytooie en die verblyf van hierdie komponente in 'n kudde verskil beduidenswaardig. Die slytooi word waarskynlik na 'n gemiddelde verblyf van sewe jaar in die kudde verkoop, terwyl die hamel op enige ouderdom verkoop kan word.

Geen inligting is beskikbaar oor die presiese komponente wat jaarliks verkoop word nie.

'n Ontleding van gegewens uit Tabel 1 dui waarskynlik op die volgende samestelling van verkope wat gemiddeld op 22 % te staan kom.

7 % tweetand hamels (21 % tweetand ooie – 14 % tweetand hamels)
7 % slytooie (1/7 van 52 % volwasse ooie)
8 % volwasse hamels (22 %–14 %)

As sowat 8 % van die gemiddelde 13 % volwasse hamels verkoop word, dui dit daarop dat die verblyf van volwasse hamels in die kuddel van korte duur is en waarskynlik binne 'n jaar nadat hulle tweetand bereik het, verkoop word.

## GEVOLGTREKKINGS

Vleis- en wolpryse het in die afgelope jare dermate vernou dat aanvaar kan word dat die boer sy merinokudde ook maksimaal sal gebruik vir 'n inkomste uit vleis.

Kuddebestuur vir vleisproduksie berus op maksimale aanteel met verkope op die vroegste ouderdom. Ideaal streef die produsent na 'n 100 % ooikudde en verkope van lammers gedurende dieselfde jaar van geboorte.

Uit die gegewens blyk dit dat die volwasse ooigetalle met sowat 12 % gedurende die afgelope twaaf jaar vermeerder het. Met slegs 21 % jong ooie beskikbaar vir verplasing van slytooie is dit duidelik dat boere, ten spyte van korrekte bestuursbesluite, nie in staat is om in hierdie stadium volwasse ooigetalle vinniger te vermeerder nie.

Om die optimale 75–80 % aanteelooie in die kudde te bereik sal boere op hierdie tydstip dus genoodsaak wees om alle vroulike diere solank moontlik in die kudde te behou en te streef na optimale reproduksie en minimale verliese van teeldiere.

Die teenwoordigheid van hamels en die ouderdomme van hierdie komponent toon dat boere waarskynlik alle bemarkbare hamels dadelik verkoop, maar dat die merinohamels dikwels eers op die ouderdom van twee jaar of meer vir die mark gereed is.

Alhoewel die merinowolskaap die grootste bron van skaapvleis vir die Republiek is, is die onbemarkbaarheid van die jong merinohamel een van die belangrikste remmende faktore in die wolbedryf. Die hamels benut vir solank as 18–30 maande weiding en bestuurskundigheid wat met groter voordeel deur aanteelooie beset kan word.

Hierbenewens ding die hamels mee met dieselfde ouderdomsgroep ooitjies wat weer tot gevolg het dat laasgenoemde op 'n later ouderdom paringsmassa bereik en dus gedurende hulle reproduksieleeftyd een lam minder lewer.

Dit skyn asof nóg die boer, nóg die navorser genoeg aandag skenk aan metodes om die jong merinohamel gouer te bemark en in dieselfde proses ook 'n jonger paringsouderdom vir die jong ooi te bewerkstellig. Die ontrafeling van hierdie knelpunt verdien myns insiens die hoogste prioriteit waar navorsing oor bestuurspraktyke ter sprake kom.

Meer navorsing moet ook gedoen word oor die optimale aantal ramme wat vir verskillende bestuurspraktyke nodig is. Intensiewe voorligting om die reeds bestaande kennis oor die benutting van ramme te versprei is uiters noodsaaklik.

## VERWYSINGS

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## INSULIN – A POSSIBLE APPETITE CONTROL MECHANISM

Most animals (and humans) "feel full" and stop eating when their stomachs are, in fact, far from full. This is an unsatisfactory state of affairs for farmers who want to rear animals quickly. Apart from wanting them to reach slaughter weight quickly, they would like to suppress feeding in cows not giving milk and carrying calves. In both situations, appetite control would greatly benefit farmers, for whom feed may amount to half of all the on-farm costs of producing milk and meat.

Dr Paul J. Wangsness, animal nutritionist at the Pennsylvania State University (University Park, Pennsylvania 16802), is seeking signals that control appetite, and one of them may be insulin production. He says "If we could boost food intake in animals by only 15 per cent, we'd have a real breakthrough". Wangsness speaks of "throttling" or accelerating the animal's food "engine", and explains that he is not interested in the maintenance level of food that keeps the "engine idling" (heart beating and lungs breathing) but, rather, on food levels above that, namely food for acceleration into the growing stage, where milk and meat are produced.

There are several thrusts in his work, to "fool" the animal into eating more. For instance, where and what the appetite signals are that supposedly signals animals to stop eating. Some believe that these are blood compounds travelling from the digestive tract to the appetite centre in the brain. In order to test this theory, Wangsness and former graduate student L.E. Chase, organised feeding trials on four Holstein steers surgically prepared for blood sampling.

Although glucose in the blood tended to decrease after feeding, and volatile fatty acids rose 15 minutes after the animals commenced eating, insulin showed the most striking change. In the blood of the calves studied, levels rose within five minutes after eating commenced. By the time the meal ended, or shortly thereafter, blood insulin dropped back to normal. (Graduate student Jean Vasilatos, of the University, hopes to support these findings in current studies with dairy cattle).

Dr Wangsness says that these findings only show an association of insulin to eating, in other words that insulin secretion changes as the animal eats. "Since blood insulin levels rose so quickly after the animal began to eat, however, it seemed possible that the hormone release was to 'stop feeding' signal". He decided to test this theory.

Firstly, he calculated the insulin dose necessary to simulate its natural rise after feeding commences. The dose was very small – from 500 to 1 000 times less than other researchers had thought to be important. Experiments were then organised, in which small amounts of insulin were injected into sheep. The reaction was quick. Almost immediately after injection, feeding slowed. Throughout feeding, the injected sheep fed 10 to 15 per cent slower than the controls.

The researcher says that he is not surprised to find that small doses "work" where large ones did not. "This kind of biological research is often based on extremes, and showing, as other researchers have done, that large doses are ineffective, does not prove that small ones are effective." "This experiment", he says, "opens up a new avenue of research, and offers a better understanding of appetite control". Wangsness adds that most scientists would like to find a natural body compound such as insulin to *control* appetite, rather than a synthetic drug. "Discovery of natural regulators, especially where tiny amounts can be used, would bypass concern about drugs and should reduce lengthy testing and approval procedures", he says.

A second line of research is based on feeding behaviour in genetically fat strains of pigs and rats. In monitoring around-the-clock feeding behaviour, animal scientists James L. Gobble (now retired), Grant W. Sherritt, and Wangsness, found that genetically fatter pigs and rats did indeed eat more. They were not only genetically disposed to fat, but they compounded the problem by higher caloric intake. "And the timing of their eating habits was distinctive", says Wangsness.

Fat animals are slower and ate less per meal, but spread their eating over the day. "They are nibblers", according to the researchers, who say that this contradicts the advice of some medical experts, who tell overweight humans to eat less at a sitting and nibble more when hunger pangs strike. "Perhaps the animals don't realise, as people are supposed to, that nibbling should help them lose weight", quip the researchers!

This research was funded by several grants, including one from the National Institutes of Health and Federal U.S. Government funds.

("Insulin – A Possible Appetite Control": Research in Agriculture, Penn State, p. 26, Progress Report 372, Pennsylvania State University, University Park, Pennsylvania 16802)

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

J. VAN HEERDEN

ABSTRACT: Van Heerden J. **The transmission of *Babesia canis* to the wild dog *Lycaon pictus* (Temminck) and black-backed jackal *Canis mesomelas* Schreber.** *Journal of the South African Veterinary Association* (1980) **51** No 2 119-120 (En) Faculty of Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

*Babesia canis* was successfully transmitted from the domestic dog to 3 wild dogs *Lycaon pictus* and 4 black-backed jackals *Canis mesomelas*. Both wild dogs and black-backed jackals showed no clinical signs or clinical pathological evidence of disease. Trophozoites of *Babesia canis* were found in peripheral blood smears from all experimental animals. The disease was also successfully transmitted from both black-backed jackals and wild dogs to the domestic dog.

## INTRODUCTION

Infection of the domestic dog with *Babesia canis* often results in overt disease. The parasite which is transmitted by *Haemaphysalis leachii* or *Rhipicephalus sanguineus* in the Republic of South Africa, invades and multiplies in red blood cells[1]. The disease manifests itself in many different ways but anaemia, icterus and bilirubinuria are the most constant clinical signs of babesiosis[1].

*B. canis* has also been successfully transmitted artificially to other Canidae, such as the silver fox, *Vulpes vulpes* and black-backed jackal *Canis mesomelas*[1,3]. Infection of splenectomized black-backed jackals caused marked anaemia and when blood from these animals was inoculated into domestic dogs 3 years later, it resulted in fatal babesiosis[3].

The parasite was also transmitted to the side-striped jackal, *Canis adustus*, wolf *Canis lupus* and wild dog *Lycaon pictus* by exposing them to ticks[1,2,3]. Neitz[3] did not describe any clinical signs of disease in the wild dog.

This report deals with experimental infection of black-backed jackals and wild dogs with *Babesia canis*.

## MATERIALS AND METHODS

Three wild dogs approximately 6 months old, 4 black-backed jackals approximately 6 months old and, as a control, an adult domestic dog (Dog 0) were used. These animals, with the exception of the domestic dog, had been reared in captivity. The jackals were reared indoors in a closed-box type stable with a concrete floor. The wild dogs were kept in a similar building but had access to a small adjoining camp. Although these animals were not reared under tick-proof conditions, regular careful inspection of the animals did not at any stage reveal the presence of ticks.

Prior to the intravenous infection of these experimental animals, blood smears were examined for the presence of parasites and haematological studies were undertaken at 2 different occasions in the case of jackals and 4 different occasions in the case of wild dogs. No *Babesia canis* trophozoites were seen.

The wild dogs were injected intravenously with 2 m$\ell$ of heparinized blood from a dog that was suffering from acute canine babesiosis. The jackals and the domestic dog were likewise injected with 2 m$\ell$ of blood from a dog in the terminal stages of babesiosis with a parasitaemia that exceeded ,04%.

Each animal was examined daily for signs of disease. Blood was collected and blood smears were taken at regular intervals during the ensuing 42 days (Table 1).

Table 1: RESULTS OF HAEMATOLOGICAL STUDIES BEFORE AND AFTER INFECTION WITH *B. CANIS*

| Days after infection | No. of exp. animal | Hb g/$\ell$ | Ht. | RCC. $10^{12}/\ell$ | WCC $10^{9}/\ell$ |
|---|---|---|---|---|---|
| 0 | Jackal 1 | 123 | 0,36 | 4,09 | 10,0 |
| | Jackal 2 | 129 | 0,42 | 4,96 | 10,9 |
| | Jackal 3 | 131 | 0,41 | 4,74 | 8,1 |
| | Jackal 4 | 130 | ,40 | 4,66 | 9,1 |
| 7 | Jackal 1 | 114 | 0,34 | 4,21 | 6,3 |
| | Jackal 2 | 131 | 0,39 | 5,16 | 8,2 |
| | Jackal 3 | 128 | 0,37 | 4,77 | 6,4 |
| | Jackal 4 | 129 | 0,35 | 4,45 | 5,8 |
| 0 | Wilddog 1 | 164 | 0,52 | 7,8 | 14,1 |
| | Wilddog 2 | 144 | 0,49 | 7,26 | 13,3 |
| | Wilddog 3 | 154 | 0,54 | 7,68 | 15,7 |
| 13 | Wilddog 1 | 149 | 0,47 | 7,08 | 14,1 |
| | Wilddog 2 | 141 | 0,45 | 6,92 | 16,9 |
| | Wilddog 3 | 121 | 0,40 | 5,88 | 7,5 |

These blood samples were used for the determination of haemoglobin content*, red cell count, packed cell volume and white cell count†. The blood smears were stained by the Quik-stain method‡ and examined under oil immersion for trophozoites of *B. canis*.

Fifteen days following infection, blood from each black-backed jackal was collected and heparinized. Two m$\ell$ of blood from each animal was then injected intravenously into a different 5-week-old domestic dog pup.

A non-infected littermate of the puppies served as a control. These puppies had been reared under tick-free conditions and were treated weekly with an insecticide and were examined daily for the presence of ticks. They were housed together under the same conditions and before and after infection, blood smears were regularly examined and haematological studies undertaken in a similar manner to that described above.

Twenty-nine days after infection of the wild dogs, 2 m$\ell$ of blood from each animal was injected intravenously into a different domestic dog pup. These puppies were approximately 5 weeks old and had been reared and maintained under tick-free conditions as described above. Blood smears were made and examined and haematological studies performed in a similar manner to those of the experimental animals. A littermate that was not infected served as a control.

*Hemoglobinometer, Coulter Electronics, Inc. Hialeah, Florida, USA.
†Coulter Counter Model FN, Coulter Electronics, INC Hialeah, Florida USA.
‡*Diff Quik* Harleco.



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Table 2: PRESENCE OF *BABESIA* PARASITES IN BLOOD SMEARS OF BLACK-BACKED JACKALS AND WILD DOGS AFTER ARTIFICIAL INFECTION

| Animal | Number used | Number positive Days after infection | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 6 | 7 | 8 | 11 | 13 | 14 | 18 | 19 | 21 | 23 | 29 | 35 | 36 | 42 | 48 |
| Black-backed jackal | 4 | 4 | – | 4 | – | – | – | 4 | Neg | 2 | 4 | 4 | 4 | Neg | – | 2 | Neg |
| Wild dog | 3 | – | 3 | – | 1 | – | Neg | – | – | – | – | – | – | – | Neg | – | 1 |

Footnote: –: not examined.

## RESULTS

Infection of wild dogs, black-backed jackals and Dog 0: Black-backed jackals did not show any clinical or haematological evidence of babesiosis (Table 1). Wild dogs did not show any clinical evidence of babesiosis. The slight drop in Hb content, red cell count and haematocrit observed in especially wild dog No. 3 was not found in subsequent haematological investigations on day 16 (Table 1). The low white cell count found in this instance is an indication of a less stressfull blood-collecting procedure and thus a normally lowered Hb-value, haematocrit and red cell count. So-called normal values in wild dogs are based on blood collected under stressfull conditions and these values usually show a high total white cell count (Table 1)[4].

Trophozoites of *B. canis* were however found in capillary blood smears of black-backed jackals and wild dogs as indicated in Table 2. Parasites were never plentiful and ranged from approximately 0,01 to 0,04% in jackals and 0,007 to 0,02% in wild dogs.

Dog 0 developed typical clinical and haematological signs of babesiosis within 6 days of infection. Capillary blood smears demonstrated many parasites exceeding an approximately level of parasitaemia of 0,04%. Specific treatment against babesiosis was given on the seventh day.

Infection of domestic dog puppies: All the puppies, except the 2 that were not infected, developed clinical symptoms of babesiosis within 4 days of infection. They became markedly anaemic and many trophozoites of *B. canis* were demonstrated in capillary blood smears (Table 3). They either died or were euthanazed in the late stages of the disease 6–7 days after having been infected.

Table 3: RESULTS OF HAEMATOLOGICAL STUDIES ON DOMESTIC DOG PUPPIES INFECTED WITH *B. CANIS*

| Pup. | Hb g/ℓ | RCC $10^{12}$/ℓ | Ht | WCC $10^9$/ℓ |
|---|---|---|---|---|
| 1 | 43 | 1,60 | 0,15 | 15,5 |
| 2 | 68 | 3,02 | 0,24 | 7,9 |
| 3* | – | – | – | – |
| 4 | 86 | 4,42 | 0,38 | 7,2 |
| 5 | 40 | 1,63 | 0,15 | 12,5 |
| 6 | 29 | 1,30 | 0,11 | 10,6 |
| 7 | 55 | 2,73 | 0,22 | 3,7 |

*Died suddenly on third day.

## DISCUSSION

Neitz & Steyn have shown that the splenectomized black-backed jackal is an efficient reservoir of *B. canis*. The present investigation confirmed that the black-backed jackal is a potential reservoir of the parasite in that

(a) following intravenous infection of entire animals (as compared with splenectomized jackals), no signs of disease were observed and

(b) blood inoculated from infected black-backed jackals to domestic dogs resulted in severe babesiosis.

It is to be noted however, that although no ticks were observed on experimental animals, they were not kept under tick-proof conditions and the possibility of existing premunity in both black-backed jackals and wild dogs can thus not be totally excluded.

The wild dogs also showed no evidence of clinical disease and blood inoculated from them to domestic dogs 29 days after infection also resulted in fatal disease. Wild dogs are thus also potential reservoirs of *B. canis*.

## ACKNOWLEDGEMENTS

My sincere thanks to the technical and medical staff of the Department of Medicine for their invaluable assistance. This study was sponsored by a grant from the University of Pretoria. The director of Nature Conservation, Transvaal Provincial Administration is thanked for permission to undertake the study.

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**CASE REPORT** **GEVALSVERSLAG**

# VOEDINGS- OF SEKONDÊRE HIPERPARATIROÏEDISME IN 'N WERPSEL DUITSE HERDERSHONDE

D.C. LOURENS

ABSTRACT: Lourens D.C. **Nutritional or secondary hyperparathyroidism in a German Shepherd litter.** *Journal of the South African Veterinary Association* (1980) **51** No. 2 121-123 (Afr. En) Department of Medicine, Faculty of Veterinary Science, University of Pretoria, PO Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Nutritional or secondary hyperparathyroidism in a litter of German Shepherd dogs is reported. The bitch lost interest in the litter 2 weeks post partum, the owner proceeded to feed the pups on a mainly meat diet (low in calcium) together with whole wheat bread (high in phosphate) until they were presented at Onderstepoort at the age of 6 weeks.

Clinically the pups showed poor growth, posterior paresis and pain on palpation of the long bones. Radiological examination revealed decreased bone density and thickness of bone cortices. A diagnosis of nutritional or secondary hyperparathyroidism was made.

The diet was corrected and in addition the pups were treated with a balanced supplement of calcium and phosphate with very good clinical response.

The pathophysiology of nutritional or secondary hyperparathyroidism as well as ricketts and hypertrophic osteodystrophy as differential diagnoses are discussed.

## INLEIDING

Voedings of sekondêre hiperparatiroïedisme is 'n veelbesproke onderwerp in die veterinêre literatuur maar ten spyte van sy hoë voorkoms en bekendheid word hierdie verslag aangebied aangesien dit wil voorkom of daar 'n wanbegrip bestaan oor 'n hoofsaaklik-vleisdieet vir karnivore.

Die toestand kom voor in groeiende honde, katte, sekere primate, laboratorium- sowel as in plaasdiere.

Die toestand kom meer algemeen in groeiende katjies voor en dit word nie algemeen besef dat die toestand ook in groeiende hondjies kan voorkom nie. Dit is 'n belangrike differensiële diagnose vir swak groei en posterior parese in jong honde.

Die patogenese van die toestand word later bespreek.

## GESKIEDENIS

Twee 6 weke-oud Duitse Herdershond-reuntjies uit 'n werpsel van agt met posterior parese is na die Departement van Geneeskunde verwys.

Twee weke post partum het die teef belang in die werpsel van agt verloor, waarna die eienaar die werpsel met vars melk, "Pro-Nutro"+, gemaalde perdevleis, volgraanbrood sowel as twee teelepels Calsuba* poeier (oplosbare gevitamineerde kalsiumvoedsel) gevoer het. Een rou eier is by elke kilogram vleis gevoeg. Die hondjies het vomeer op hierdie dieet en die eienaar het die melk uit die dieet verwyder.

Op vyf weke ouderdom het een van die hondjies 'n spontane fraktuur van die linker femur ontwikkel. Die been is in gips geplaas maar na twee weke moes genadedood weens nekrose van die been toegepas word.

Op ses weke ouderdom het die hele werpsel posterior parese ontwikkel en is twee reuntjies na die Departement van Geneeskunde verwys.

## KLINIESE ONDERSOEK

Die normale gewig vir Duitse Herdershondjies van hierdie ouderdom is 15 kg[9]. Hierdie hondjies was egter heelwat kleiner en het 7,5 kg geweeg. Beide hondjies was vriendelik en wakker van geaardheid maar het geweier om enigsins te beweeg. Wanneer beweging egter wel plaasgevind het, was die posterior parese uitgesproke.

Palpering aan die skelet en veral van die agterbene was uiters pynlik en hulle het getjank indien 'n vinnige beweging in hulle rigting gemaak is. Met palpering het die een hondjie 'n midskagkallus van die linker femur getoon.

## SPESIALE ONDERSOEKE

1. Feses-, urien- en hematologiese ondersoeke het geen abnormaliteite getoon nie.
2. Bloedchemie.

| | Normaal[2] | Pasiënt A | Pasiënt B |
|---|---|---|---|
| Kalsium | 4,6–6,1 m mol/ℓ | 5,45; 4,7 | 5,8; 5.6 |
| Fosfaat | 1,3–2,9 m mol/ℓ | 2,6; 2,8 | 3,0; 2,8 |
| SAP (Serum alkaliese fosfotase) | 50–122 mU/mℓ | 244; 300 | 640; 304 |

Een of twee bepalings ten opsigte van kalsium en fosfaat is van geen waarde nie aangesien kompenserende meganismes baie kompleks is en gewoonlik lank reeds in werking is. Sodoende word normale bloedkalsium- en bloedfosfaatwaardes verkry[2,5].

SAP-vlakke neig om te styg met verhoogte osteoblastaktiwiteit maar die verhoogde vlakke is nie spesifiek nie. SAP-vlakke verhoog normaalweg in

+Pro-Nutro: Food Corporation, Randburg.
*Calsuba: Group Laboratories S.A. (Edms) Bpk, Wrenchweg 21, Isando, Transvaal.



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groeiende diertjies en verhoogde SAP-lesings kan ook te wyte wees aan lewerpatologie[2].

3. Radiologie (Fig. 1 en 2).

Radiologiese ondersoek het die volgende aan die lig gebring: Algemene vermindering in digtheid van been met verdunning van beenkorteks. Verwyding van beenmurgholte met slegs baie dun oorblywende kortikale been. Let op dat die vorm van die metafise en begrensing met die epifise onveranderd is. As gevolg van beenswakheid kan sekondêre prosesse soos frakture, (Fig. 2) verbuiging en kolaps intree.

**Fig 1** Algemene vermindering in beendigtheid met verdunning van beenkorteks met gevolglike verwyde beenmurgholte

**Fig 2** As gevolg van beenswakheid tree sekondêre prosesse soos frakture in

## BEHANDELING

Beide hondjies is behandel deur 8 g ICD* granules by 'n gebalanseerde dieët te voeg.

ICD-granules bevat 'n gebalanseerde verhouding van kalsium, fosfaat, Vit D en ook laktose wat intestinale absorpsie van kalsium verhoog. Die hondjies se beweging is ook beperk deur hulle op hok te hou. Die gebreekte femur van die een hondjie is nie spesiflek behandel nie.

*ICD Granules, Glaxo Allenburys (Edms) Bpk, Manchesterweg, Wadeville, Transvaal.

Een week na die aanvang van die behandeling het 'n merkbare kliniese verbetering reeds ingetree en na twee weke het die radiologiese beeld reeds 'n toename in beendigdheid getoon.

Die res van die werpsel is aan dieselfde behandeling onderwerp en weke later het die hondjies klinies volkome herstel.

## BESPREKING

'n Diagnose van voedings- of sekondêre hiperparatiroïedisme is gemaak op grond van:

1. 'n Dieët wat hoofsaaklik uit vleis bestaan en wat laag in kalsium is. Die diere het ook heelwat volgraanbrood ontvang wat ryk aan fosfaat is. Alhoewel bykomstige kalsium gevoer is, was dit heeltemal onvoldoende.
2. Kliniese simptome.
3. Radiologiese beeld.
4. Reaksie op behandeling.

Differensiële diagnose wat oorweeg is, was ragitis en hipertrofiese osteodistrofie.

### Ragitis

Ragitis is 'n metaboliese siekte van jong, groeiende diere wat gewoonlik te wyte is aan onvoldoende plasmavlakke van vitamien D. Ragitis is 'n rariteit in die hond en kat. Alleenlik die mens, marmot en aap benodig eksogene vitamien D[3]. Ragitis kom waarskynlik slegs voor in diere waar die blootstelling aan sonlig minimaal is. Sonlig is essensieël vir die sintese van vitamien D vanaf cholesterol[3].

Klinies word die toestand gekenmerk deur vertraagde groei, apatie, spierswakheid, vergrote groeiplate en later verbuiging van langbene. Die kraakbeenmatriks kalsifiseer nie genoegsaam nie en degenerasie van groeiende kraakbeen vind nie plaas nie, met gevolglike onordelike kalsifisering en onreëlmatige oorgroei van kraakbeen. Dit word verder gekenmerk deur 'n wye epifiseale kraakbeenplaat. Die bene toon 'n onvermoë om die dier se toenemende massa te dra en dit lei tot vergroting van die gewrigte deurdat die sagte metafise meegee en die hele been begin buig.

'n Radiologiese ondersoek toon gewoonlik wye radiodeurlaatbare groeiplate, onvoldoende mineralisasie van been, verbuiging van die lang bene, uitbulting van die metafise en wye, oneweredige epifiseale en metafiseale grense[3,7].

### Hipertrofiese osteodistrofie[1,3,4]

(Skeletale skeurbuik, Möller-Barlowsiekte, Hipovitaminose C)

Bogenoemde is 'n sindroom in jong groeiende hondjes van 4–6 maande. Dit kom hoofsaaklik voor in die vinnig-groeiende groot rasse en word gekenmerk deur akute pyn en geswelle van die distale metafise van lang bene. Die honde verkies gewoonlik om te lê en toon dikwels wisselende koorsreaksies.

Waarskynlik is die toestand te wyte aan oormatige voeding van minerale (veral kalsium), fosfaat en vitamines (veral Vit D). Die hondjies toon ook dikwels lae bloedaskorbiensuurvlakke.

Oormatige hoeveelhede minerale word neergelê in die metafiseale gebiede waar endochondrale osteogenese die aktiefste is (distale radius, ulna en tibia). Radiologiese ondersoeke toon verhoogde digtheid van die

metafise veral distaal, oormatige vergroting van die metafise, subperiosteale bloedings gevolg deur periosteale hiperplasie en subperiosteale en ekstraperiosteale beenneerlegging.

### Voedings- of sekondêre hiperparatiroïedisme

Bogenoemde is te wyte aan 'n verhoogde sekresie van paratiroïed-hormoon as gevolg van wanvoeding wat lei tot versteurde kalsiumfosfaatbalans[3]. Hipokalsemie is die stimulus vir die ooraktiwiteit van die paratiroïedkliere en kan verskeie oorsake hê[3]. 'n Dieët laag in kalsium, soos een wat hoofsaaklik uit vleis bestaan, of 'n dieët met oormatige fosfaat met normale of lae kalsiumvlakke (soos in die geval van graankos) en laastens onvoldoende Vit $D_3$.

Vleis het 'n baie lae kalsiuminhoud (9 mg per 100 g)[3] [9] en 'n baie ongewensde kalsium-fosfaatverhouding van 1:22.

Dit volg dan hieruit dat diere wat hoofsaaklik op 'n vleisdieët gehou word, hipokalsemie ontwikkel wat dan lei tot verhoogde paratiroïed-aktiwiteit met gevolglike verhoogde osteoklastiese resorpsie van been, swak mineralisasie van osteoïed wat in oormaat gevorm word deur osteoblaste, verhoogde bloedkalsium- en fosfaatvlakke, verhoogde renale uitskeiding van fosfaat en laastens verhoogde intestinale absorpsie van kalsium.

Inname van oormatige hoeveelhede fosfaat lei tot verhoogde absorpsie uit die dermkanaal en gevolglik verhoogde bloedvlakke wat dan lei tot hipokalsemie wat op sy beurt verhoogde paratiroïedsekresie tot gevolg het[3].

Dit kan genoem word dat honde wel van nature vleisvreters is maar in die natuur leef wilde karnivore nie net van spierproteïene nie. Tydens verorbering van 'n karkas vreet hulle ook skeletweefsel, klierweefsel en interne organe[8].

In die tye waarin ons leef kry ons nog steeds die probleem tsv wetenskaplike formulering van rantsoene. Wanopvattings by eienaars oor karnivore soos hierbo genoem, onvoldoende raadgewing aan eienaars en selfs die advertensie van 'n sogenaamde "all meat diet" kan geblameer word dat ons vandag nog steeds gevalle van voedings- of sekondêre hiperparatiroïedisme kry[6 8].


## BEDANKINGS

Prof C J Roos word bedank vir interpretasie van die X-straalfoto's, Mev H Rothman vir die neem van foto's en mev H J van Aarde vir die tik van hierdie verslag.


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## BOOK REVIEW

## BOEKRESENSIE

### THE VETERINARY ANNUAL

### TWENTIETH ISSUE
### EDITORS: G.S.G. GRUNSELL and F.W.G. HILL
### John Wright and Sons Ltd, Bristol, England 1980 pp xvi and 319. Price £13,00
### (ISBN 0 85608 028 4).

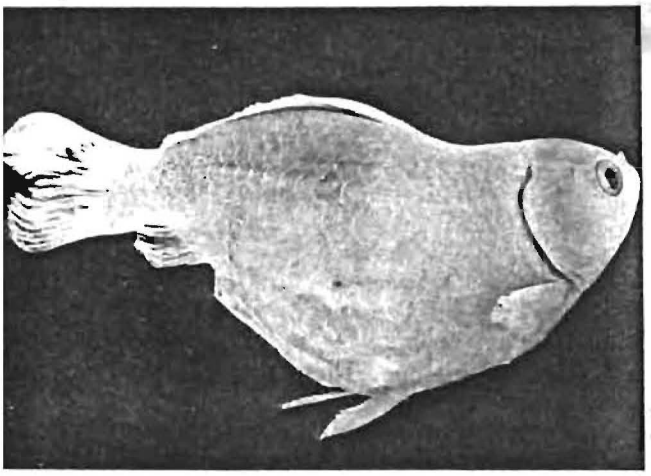
This book which by now should be well known to most of the veterinary profession has now appeared annually for the last 20 years. This in itself is an indication as to its usefulness and popularity. The present volume is presented in the same tradition as its predecessors, i.e. the latest literature of certain selected topics is briefly reviewed by authorities in the relevant fields. This assists one to keep abreast of current knowledge and trends.

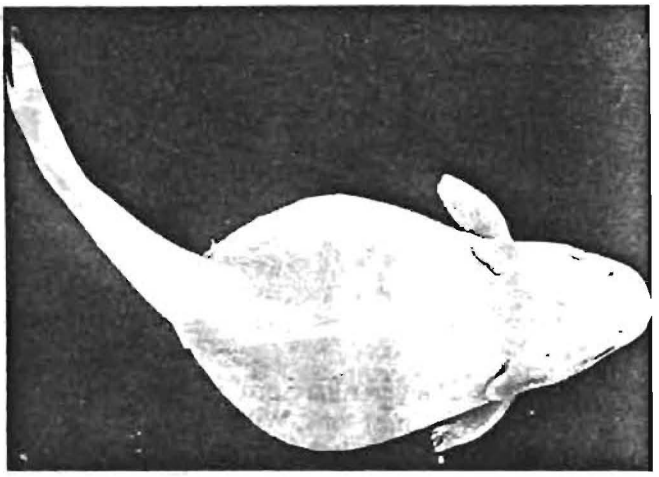
The present volume includes chapters on Reproduction and infertility; Reviews of animal husbandry; Meat inspection in England and Wales; Clinical features and laboratory evaluation of canine liver disease; Stomach, respiratory, prostatic and muscle diseases of dogs; Biological behaviour of mastocytomas and melanomas in dogs; Canine heartworm disease; Chloramphenicol toxicosis in dogs and cats; Mycoplasms of the cat; Preanaesthetic medication in small animals; Immunoglobins in the foal from the practitioner's viewpoint; Equine carpal fractures; Stinghalt; Rotavirus in farm animals; Cerebrocortical necrosis; Calf pneumonia; Infectious bovine rhinotracheitis; Trace element deficiencies in Scotland; Abdominal distension in cattle; White line disease of the bovine foot; Pasteurellosis in sheep; Teat deficiencies in pigs; Aujesky's disease; Porcine reproductive failure; Pig feed additives; Navel bleeding in piglets; Role of veterinarian in the turkey industry.

**RCT**

# THE ULTIMATE OVARIAN CYST?

G.F. BATH*





The goldfish illustrated was one of about 15 living in a smallish open air pond and fed on commercial fish food.

It was the only fish in 5 years to show any sign of abdominal enlargement. At first it seemed that it might just be spawning but as weeks dragged into months it was obvious that pathological changes were under way. The abdomen gradually enlarged over about a year and during this time it became progressively more difficult for the fish to swim and remain upright. In the latter stages the fish could be seen floating belly up just under the water but would always right itself and swim away if disturbed. It continued to feed well until the time that euthanasia was decided on. At that stage a neoplasm was suspected.

On opening the fish, neither a neoplasm nor ascitic fluid were present. Instead a huge cyst of clear watery fluid was found attached to the ovary. The fish and viscerae minus fluid from the cyst had a mass of 33 g while the cyst fluid was approximately 18 m$\ell$ in volume. This means that the cyst made up about 35% of total body mass or over 50% of the normal body mass – a percentage only possible in an aquatic animal.

From the literature[1,2] on diseases of fish consulted, no description of a similar condition could be found. Clinically it might be confused with dropsy but there was no emaciation, scale protrusion or exophthalmos. Skeletal malformation seen in the lateral view was probably due to continual pressure of the abdominal contents on the spine.

The cause of the massive cyst remains speculative but taking into account the slow development, the lack of other signs of ill health and the absence of further cases before or since this one, it seems that it was not nutritional or infectious but rather a chance aberration of ovarian function.

*Regional Veterinary Laboratory, Private Bag X528, 5900 Middelburg.

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# IN MEMORIAM

## PROF DR W.O. NEITZ

On 18 August 1979, the veterinary profession in South Africa lost one of its greatest sons, Wilhelm Otto Neitz.

He was born on 17 November 1906 at Potgietersrus in the Northern Transvaal. His early awareness of the importance of tick-borne and other bushveld diseases of livestock made the study of veterinary science at the University of Pretoria's Faculty of Veterinary Science at Onderstepoort, a natural choice and he was awarded the BVSc degree in 1929.

A brilliant research career followed on his appointment in 1930 to the Veterinary Research Institute, Onderstepoort, where he worked until his retirement in 1971. It soon transpired that research was his passion and his dedication to his work and his concern for his experimental animals were legendary. His career was marked by many notable achievements, particularly in the field of infectious diseases. He was a very versatile scientist and achieved fame not only as a protozoologist but also in the fields of virology and acarology.

A series of highlights in his studies are proof of his ability and versatility. His work on the immunity in bluetongue led to the discovery of the multiplicity of strains of bluetongue virus. This was submitted as a thesis for which he was awarded a DVSc degree by the University of Pretoria in 1945.

Prof. Neitz developed the blood vaccine against heartwater which, with minor modifications, is still in use. He also discovered the efficacy of certain sulphonamides against heartwater, thereby showing for the first time that this highly fatal disease can be cured. He successfully conducted studies on the immunology of heartwater which formed the basis for interpretation of the epizootiology and implementation of prophylactic measures.

He is particularly well remembered for his work on theileriosis. His discovery of the schizonticidal effect of tetracyclines on *Theileria parva* is currently being used with great success in the infection-cum-treatment method of immunization in East Africa against East Coast fever. He discovered that Corridor disease was caused by *Theileria lawrencei* and that buffalo served as carriers whereas cattle were not a source of infection for the vector tick *Rhipicephalus appendiculatus*.

He was also involved in the discovery of a fatal form of theileriosis in antelope. These studies were subsequently extended elsewhere in Africa and have led to further clarification of the epizootiology of the theileriosis.

One of his most interesting discoveries was that sweating sickness of cattle was transmitted by the tick *Hyalomma truncatum*, thereby solving the enigma that had puzzled other scientists for so many years.

The majority of his discoveries were of practical importance and of value not only to the South African veterinarian and farmer but also to his counterpart elsewhere in Africa.

Prof. Neitz later served as Professor of Protozoology and Virology at the Faculty of Veterinary Science from 1948 to 1957 and, after the curriculum was changed, as Professor of Protozoology until 1967. Many South African veterinarians studied under him and his tremendous contribution to veterinary education at both graduate and post-graduate levels has put his distinctive stamp on veterinary science as it is practised in this country. He was an inexhaustible source of information in his specific field of interest and, like the ancient Greek philosophers, took pleasure in imparting his knowledge to interested colleagues, visitors and his own staff.

His research work culminated in 133 publications which appeared from 1927 to 1971. Each article was meticulously written and rewritten several times in his neat handwriting before being finally submitted to the typists. Many of his publications were comprehensive reviews and much sought-after by other scientists. These, together with his undoubted distinction as a scientist, won for him local and international recognition on a level which few have managed to equal.

He received several coveted awards and other forms of recognition, as indicated by the following:

1. The Senior Captain Scott Memorial Medal of the S.A. Biological Society in 1954.
2. The "Havengaprys vir Geneeskunde" of the S.A. Akademie vir Wetenskap en Kuns in 1957.
3. The degree "Dr. Med. Vet. honoris causa" by the Tierärztliche Hochschule, Hannover, Federal Republic of Germany in 1963.
4. The "South Africa Medal for 1970" of the S.A. As-

sociation for the Advancement of Science in 1970.
5. The first "Gold Medal of the S.A. Veterinary Association" in 1971.
6. The "Elsdon-Dew Medal" of the Parasitological Society of Southern Africa in 1975.

He also served on several South African and international bodies and societies: He was President of the S.A. Biological Society in 1944. He served as consultant to the FAO on African swine fever and on the Expert Panel of the FAO/OIE on tick-borne diseases from its inception in 1956 until 1964. He was leader of the discussions on ovine and caprine rickettsial diseases at an OIE meeting in Paris in 1968 and served on the Committee of the List of Animal Diseases of the International Veterinary Congress. He was invited to act as chairman of the subsection Trypanosomiasis at the World Veterinary Congress. Mexico, in 1971.

On several occasions he conducted seminars on tropical diseases on invitation by the Free University of West Berlin. Finally, he was visiting Professor at the Federal Rural University in Brazil from his retirement from Onderstepoort in 1971 until his death. Here he served as a wise and greatly revered teacher to post-graduate students in parasitology.

He died in harness, as could have been expected.

Prof. Neitz was unmarried. To his surviving brothers and sisters and other relatives we extend our sincere condolences.

## BOEKRESENSIE — BOEK REVIEW

### AN ATLAS OF SURGICAL APPROACHES TO THE BONES OF THE DOG AND CAT

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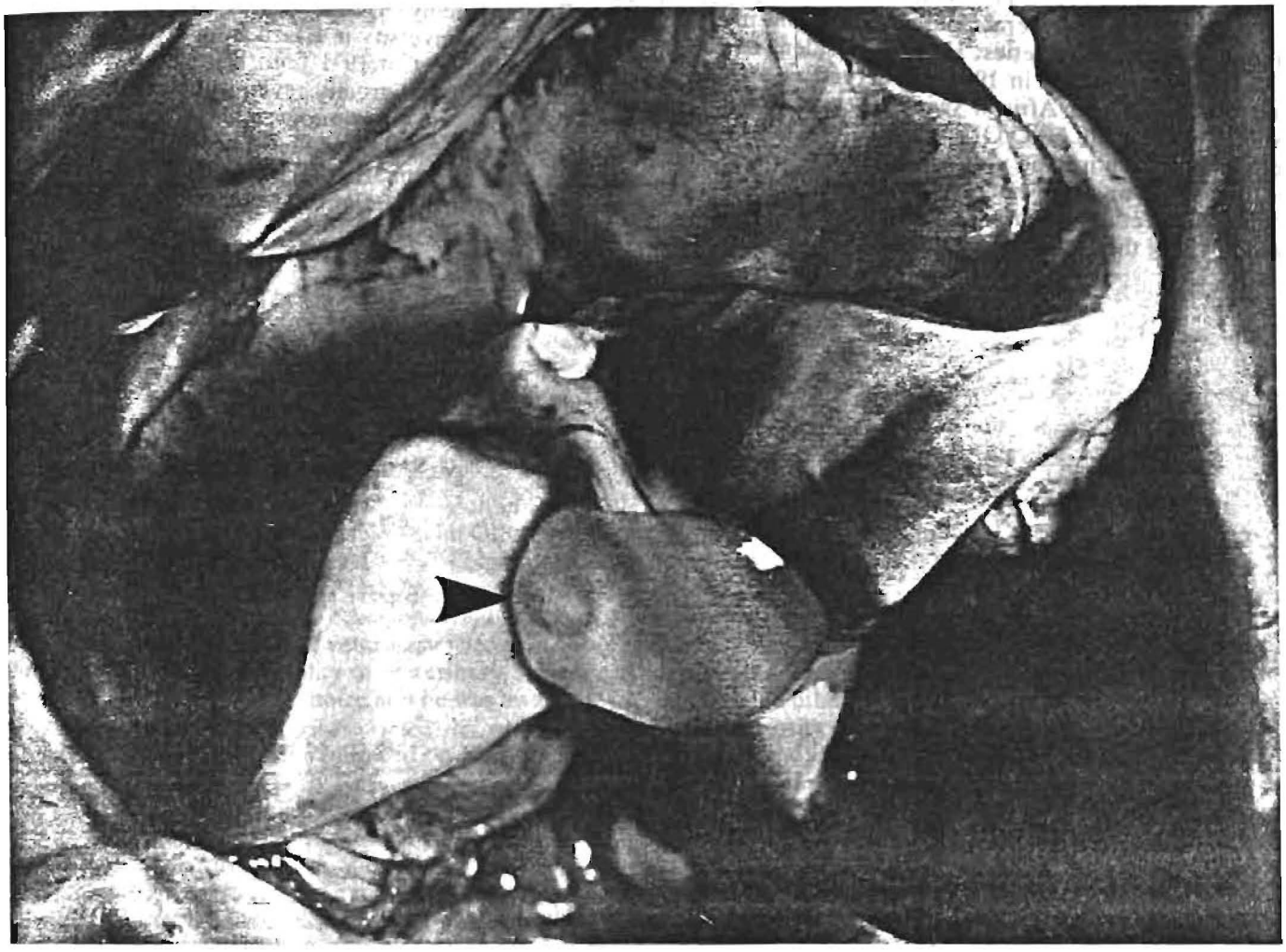
Die tweede uitgawe van hierdie merkwaardige atlas bevat aansienlik meer materiaal as die eerste. Die toename in bladsye vanaf 132 in die eerste tot 202 in die nuutste uitgawe tesame met die vergrote formaat is 'n aanduiding van die hoeveelheid addisionele materiaal wat in laasgenoemde opgeneem is.

Die eerste afdeling van die boek word gewy aan algemene oorwegings. Aseptiese tegniek word in groter besonderhede behandel en die tweede uitgawe sluit ook 'n kort oorsig van die anatomie van die ledemate en nek in. Dit word gevolg deur die benaderings tot die bene van die kop en daarna die werwelkolom. Die skapula en skouergewrig word as 'n afsonderlike afdeling behandel waarna die voorbeen volg. So word ook die bekken en heupgewrig in een afdeling saamgevat met die agterbeen as die laaste hoofstuk in die boek.

Sedert die verskyning van die eerste uitgawe in 1966 het ortopediese chirurgie van die hond en kat met rasse skrede vooruitgegaan. In die nuwe uitgawe is aandag aan hierdie vooruitgang en die beoefening van tegnieke gewy deurdat veral die toegang tot bene vir die aanbring van plate en skroewe in besonderhede behandel word. Die blootlegging van gewigte vir rekonstruktiewe chirurgie geniet ook meer aandag as voorheen. Die benaderings tot die verskillende liggaamsdele word verder aangevul deur die insluiting van benaderings van bekende chirurge. Dit is jammer dat die nuwe illustrasies wat in die Atlas opgeneem is nie ook deur Dr Greeley geteken kon word nie. Daar is 'n duidelike verskil tussen die illustrasies van die eerste uitgawe en die wat nuut in die tweede uitgawe opgeneem is. Die nuwe illustrasies deur F.D. Giddings is egter van baie hoë gehalte en doen geensins afbreuk aan die boek nie.

Hierdie atlas is sekerlik een van die mees praktiese en onontbeerlike handleidings beskikbaar vir die veearts met 'n belangstelling in ortopediese chirurgie. Die illustrasies tesame met die kort en duidelike teks maak dit baie maklik om die benadering tot 'n spesifieke deel te volg. Dit is die soort van boek wat herhaaldelik geraadpleeg kan word deur die onervare sowel as die ervare chirurg. Dit is onontbeerlik vir die opleidings van studente in operatiewe chirurgie. Dit behoort in besit van elke privaatpraktisyn met belangstelling in ortopediese chirurgie van die hond en kat te wees. Die atlas kan dus met vrymoedigheid aanbeveel word.

**DGS**



## SEREBRALE SISTISERKOSE BY 'N HOND

Die *Cysticercus cellulosae* (deur 'n pyl aangedui) is in die regter laterale serebrale ventrikel van 'n sesjaar oue Bulldog gevind. Die sist was vry in die ventrikel beweeglik en 'n ligte mate van unilaterale hidrokefalus was teenwoordig aan dieselfde kant.

Volgens bewering het die hond vir etlike maande voor die na-doodse ondersoek sporadiese grand mal epileptiese aanvalle getoon. Oorspronklik is die epileptiese aanvalle opgemerk slegs as die dier vir 'n tyd lank geslaap het. Is dit dus moontlik dat 'n tydelike obstruksie van die vloei van serebrospinale vog deur die foramen van Monro plaasgevind het, nadat die dier vir 'n tyd lank gelê het, moontlik met sy kop in 'n spesifieke houding – in ander woorde, dat die sist soos 'n tipe koeëlklep reageer het deur die aksie van die vloei van serebrospinaalvog en gravitasie – en dat die vinnige opbouing van intraventrikulêre druk vir die simptome verantwoordelik was? Die aanvalle het geleidelik meer gereeld voorgekom en was van erger graad. Genadedood is uiteindelik tydens status epilepticus toegedien.

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## CEREBRAL CYSTICERCOSIS IN A DOG

The *Cysticercus cellulosae* (indicated by an arrow) was found in the right lateral cerebral ventricle of a six year old Bulldog. The cyst was freely moveable in the ventricle and a mild unilateral hydrocephalus was present on the same side.

The dog allegedly had sporadic grand mal epileptic fits for some months preceding the autopsy. Initially the epileptic fits only occurred some time after the animal had been asleep. Is it therefore possible that a temporary occlusion to the flow of cerebrospinal fluid through the foramen of Monro took place after the animal had been recumbent for a period, possibly with its head in a particular position – in other words, that the cyst acted as a type of ball-valve whose action was affected by the flow of cerebrospinal fluid and gravity – and that the sudden build-up of intraventricular pressure was responsible for the symptoms? The fits gradually became more frequent and severe in nature. Euthanasia was eventually performed during a status epilepticus.

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## *ACTINOBACILLUS SEMINIS* INFECTION IN SHEEP IN THE REPUBLIC OF SOUTH AFRICA. I. IDENTIFICATION OF THE PROBLEM

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ABSTRACT: Van Tonder E.M. 1979. *Actinobacillus seminis* infection in sheep in the Republic of South Africa. I. Identification of the problem. *Onderstepoort Journal of Veterinary Research*, 46, 129-133 (1979).

A clinical palpation and semen smear examination of 647 rams submitted to the Regional Veterinary Laboratory during 1967 revealed that 42 (6,5%) of these animals had clinical epididymitis or orchitis, 6 (0,9%) showed other types of genital lesions and 98 (15,1%) suffered from subclinical genital infection. *A. seminis* and *A. seminis*-like organisms were isolated from semen specimens of 18 out of 35 rams with clinical epididymitis or orchitis, 25 out of 33 rams with subclinical infection and none out of 13 rams which showed no neutrophils in their semen.

On 4 stud farms where Elberg Rev. 1 vaccine was meticulously applied and the complete absence of *Brucella ovis* infection was established, of a total of 327 rams examined, 10 (3,6%) were found to be clinically and 72 (22,0%) subclinically affected. *A. seminis* was isolated from 5 out of 6 of these rams with clinical lesions and 10 out of 15 of those which showed evidence of subclinical infection.

## *ACTINOBACILLUS SEMINIS* INFECTION IN SHEEP IN THE REPUBLIC OF SOUTH AFRICA. II. INCIDENCE AND GEOGRAPHICAL DISTRIBUTION

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ABSTRACT: Van Tonder E.M. 1979. *Actinobacillus seminis* infection in sheep in South Africa. II. Incidence and geographical distribution. *Onderstepoort Journal of Veterinary Research*, 46, 135-140 (1979).

To obtain information on the incidence and distribution of *Actinobacillus seminis* infection in the Republic of South Africa, a clinical and serological survey was carried out on 409 farms situated in 29 districts. All rams submitted for certification to the Regional Laboratory from 1/1/69 to 31/1/74 were included in a separate investigation. These particular rams represented different breeds and originated from farms in over 48 districts. Examinations were also carried out on all rams on 11 stud farms in the Middelburg and adjacent districts with a high incidence of epididymitis, despite regular immunization with Elberg Rev. 1 vaccine.

These investigations confirmed that genital infection of rams still presents a major problem in the main sheep breeds and the main sheep farming areas of South Africa. A high incidence of infection with *A. seminis*, an organism which appears to be the most important one associated with genital infection in this country, was also established. Genital infection due to *A. seminis* is geographically also very widespread.

## *ACTINOBACILLUS SEMINIS* INFECTION IN SHEEP IN THE REPUBLIC OF SOUTH AFRICA. III. GROWTH AND CULTURAL CHARACTERISTICS OF *A. SEMINIS*

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ABSTRACT: Van Tonder E.M. 1979. *Actinobacillus seminis* infection in sheep in the Republic of South Africa. III. Growth and cultural characteristics of *A. seminis*. *Onderstepoort Journal of Veterinary Research*, 46, 141-148 (1979).

Bacteriological tests were done on a large number of different strains of *Actinobacillus seminis* and also, repeatedly, on the same culture or on different cultures taken periodically from the same donor animal. These tests were also applied to strains of *A. seminis* representing different serological types, which in turn were compared with strains of *Brucella ovis*.

The tests as applied proved that *A. seminis* strains have defined, morphological, staining, cultural and biochemical properties, although they can generally be regarded as biochemically inactive. Growth was greatly enhanced on media enriched with blood or serum and also more luxuriant when incubated in a carboxophilic atmosphere. Nitrate reduction was found to be a variable characteristic, as it was more often negative, while weakly positive and negative reactions for hydrogen sulphide production were encountered with equal frequency.

On the basis of their bacteriological properties, the strains representing the different serological types can be divided into 2 groups. Strains belonging to the first of these groups conform to the earlier description of *A. seminis* by Baynes & Simmons (1960) and are usually catalase positive and oxidase negative, while those in the second group more closely resemble *Histophilus ovis* described by Roberts (1956), and produce variable reactions on the catalase and oxidase tests.

Although growth did occur aerobically and was more luxuriant in a carboxophilic atmosphere in all strains, it was always much slower for strains resembling *H. ovis*. Similarly, the growth produced by these strains was poorer and more irregular on ordinary nutrient media and, although greatly enhanced and more regular in all strains on enriched media, it was again much slower for these strains. In all stages of development, the colonies of strains similar to *H. ovis* were always slower and more transparent in appearance, and tended to remain low convex and undifferentiated. Packed organisms of these strains were light yellow (lemon) in colour in contrast to strains resembling *A. seminis*, which had a greyish-white appearance.

*A. seminis* and *B. ovis* can clearly be distinguished on their morphology, Stamp staining reaction on both semen and culture smears, colonial morphology, the delayed colony development of *B. ovis* and sensitivity to dyes and antibiotics.