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Bent-leg syndrome in a lamb

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Contents • Inhoud

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A CUTANEOUS HORN IN A CAT

A 13-year-old female housecat presented for clinical examination, had a growth on the lower right hand side of the neck. The lesion had been present for more than a year. The cat later died of unrelated causes. The growth on the neck was a cutaneous horn (Fig. 1) which measured 6 x 1,5 cm and hung loosely from the skin. Histological examination revealed hyperkeratosis of the epidermis and much mature collagen was visible in the dermis. The sections were stained, using the Masson trichome technique.

Cutaneous horns occasionally occur in any type of domestic animal, although they are more common in ruminants. The cause of such horns my be chronic irritation; they may originate from papillomas, basal cell tumors, squamous cell carcinomas, or other keratoses, or they may be of unknown origin. Multiple cutaneous horns on the footpads of cats have been reported in association with feline leukaemia viral infection. A virus was cultured from the horn, and type C viral particles were seen electronmicroscopically in the lesion.

Cutaneous horns may be single or multiple, and occur in any species, breed, sex, age, or site. Their sizes vary considerably. Histologically, extensive, compact laminated hyperkeratosis is observed.

Histopathologists recommend an examination of the base of the horn in order to establish the underlying cause, but in this case nothing was revealed.

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Fig. 1: Cutaneous horn (arrow) in a 13-year-old female cat

BOVINE BRUCELLOSIS IN THE HIGHVELD REGION: EFFECT OF CALFHOOD VACCINATION

After 10 years of compulsory heifer calf vaccination with strain 19 (S 19) vaccine, the incidence of brucellosis in dairy herds, in the State of California, decreased from 17% to 1,7%[3]. Based on this type of experience, compulsory vaccination of heifer calves, in the Republic of South Africa with a similar vaccine, was proclaimed in Government Gazette R 2252 of 13 December 1968. According to this proclamation, all heifer calves between the ages of 3 and 10 months were to receive a single dose of S 19 vaccine. As about 18% of dairy herds in the Highveld region reacted to the milk ring test[1] 20 years later, the reliability of the vaccination programme, as applied in this region, was questioned.

In order to test the efficacy of this programme, a questionnaire, probing the matter of calfhood vaccination, was circulated to all dairy farmers in the northern Orange Free State (NOFS). Questionnaires applying to herds which had been blood tested during the period April 1987 to March 1988, were selected for further investigation. The number of reactors found in the first blood test of each of these herds during the above period, was taken as the incidence of the disease.

Nothwithstanding this proclamation, only 32% of respondents indicated that they had practised calfhood vaccination for 10 years or more. At least 13% of respondents had not vaccinated their heifer calves at all. On farms where heifer calves had not received S 19 vaccine, the incidence of reactors was 4,9% (Table 1). This figure was significantly higher than the 1,6-1,8% of reactors found on farms where S 19 vaccination had been administered regularly. The number of reactors on farms where vaccination had been practised for 10 years or longer, did not differ significantly from those on farms where heifer calves had received their dose of S 19 vaccine for fewer than 10 years.

Table 1: **The effect of calfhood vaccination on the incidence of brucellosis.**

Calfhood vaccination for	Number of reactors	Number of animals tested	Percentage of reactors
> 10 years	39	2 165	1,8
< 10 years	150	9 322	1,6
not vaccinated	76	1 562	4,9
Total	265	13 049	2,0

It is generally agreed that S 19 vaccination alone cannot eradicate brucellosis from a cattle population, but that it can be used effectively, together with other measures to lay the groundwork for eradication[3]. The effect of vaccination on some of the dairy farms in the NOFS, however, compared well with results obtained overseas[2, 3]. This indicates that heifer calf vaccination with S 19 must be regarded as the backbone of brucellosis eradication in this area. As a reasonably representative number of dairy farms were included in this survey, one can assume that a significant percentage of the local dairymen still do not comply with the conditions of proclamation R 2252.

Calfhood vaccination with S 19 should therefore, not be left to the whims of the farming community, but should involve active intervention by the Division of Veterinary Services.

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WURMWEERSTANDPROEF

Weerstand teen wurmmiddels is vermoed by 'n Dorper-kudde in die Venterstad distrik (K.P.). Die eienaar koop nie skape in nie en maak al jare lank van sy eie aanteelprogram (behalwe ramme) gebruik. Sy dragtige ooie word 'n maand voor lamtyd na besproeide grasklawerweidings gebring waar die lammers ook grootgemaak word. Die grasklawerweidings is nou al 13 jaar lank in gebruik. Rotasie het tot gevolg dat die skape elke 2 maande terugkom na dieselfde weiding. Die lammers word vanaf 1,5 maande ouderdom maandeliks gedoseer met 'n verskeidenheid van wurmmiddels wat hoofsaaklik aan die bensimidasool-groep middels (albendasool) behoort. Seponver en rafoksanied is ook in die somermaande toegedien. Ten spyte van die goeie voeding en gereelde doserings het die lammers swak gegroei. 'n Nadoodse ondersoek op 'n aantal skape het op 'n besmetting van *Ostertagia* sp en *Trichostrongylus* sp gedui.

Met bogenoemde agtergrondinligting is 'n proef uitgevoer met wurmeiertelling-reduksie as basis. Die rekenkundige gemiddeld van elke groep lammers se wurmeiertellings is telkens bereken[1]. In die proefneming is 60 Dorperlammers van die eienaar in 5 groepe van 12 ingedeel sodat die groepe massagewys redelik eenvormig was. Mismonsters is van elke lam versamel vir ontleding onmiddellik voor dosering. Doserings is volgens voorskrifte op die etiket van elke middel toegedien. Dubbel dosisse dui dus 2 keer die aanbevole dosis aan. Sewe dae later is daar weer van elke skaap mismonsters geneem. Tabel 1 dui die gemiddelde aantal wurmeiers per gram mis (EPG) voor en na dosering aan sowel as die persentasie vermindering in gemiddelde wurmeiertelling van elke groep[1].

Uit die tabel kan afgelei word dat daar omtrent 'n algehele weerstand teen albendasool (teen enkeldosis) is. Die dubbeldosis het egter wel 'n 66% reduksie van wurmeiertelling tot gevolg gehad. Waar wurmmiddelweerstand 'n algemene probleem is, is dubbeldosisdoserings of die gelyktydige gebruik van 2 soorte middels al in die praktyk toegepas[2]. Terselfdertyd is morantel en ivermektien steeds baie effektief (97% reduksie). Met die wurmeier-reduksietoetsmetode kan wel nie bepaal word watter spesifieke rondewurm weerstand opgebou het teen die wurmmiddels nie. Dit is egter 'n handige metode waarmee die praktiserende veearts aan sy kliënte kan toon of daar op hulle plase rondewurms is met weerstand teen een of meer wurmmiddels.

Tabel 1: **Die gemiddelde aantal wurmeiers per gram mis voor en na dosering sowel as die persentasie vermindering in gemiddelde wurmeiertelling na behandeling met albendasool, morantel tartraat en ivermektien**

Groep	Middel	EPG voor Dosering (SD)	EPG na Dosering (SD)	% Reduksie
1	BZ (2ml 10 kg^{-1})	2 000 (2 470)	1 725 (2 006)	13,8
2	2 x BZ	2 180 (2 108)	733 (1 600)	66,3
3	MT (2,5ml 10 kg^{-1})	4 625 (7 108)	100 (75)	97,8
4	2 x MT	1 100 (765)	25 (35)	97,7
5	IVM (2,5ml 10 kg^{-1})	8 100 (15 927)	171 (221)	97,9

BZ = Albendasool
MT = Morantal tartraat
IVM = Ivermektien
SD = Standaard deviasie
2 x = Dubbeldosis

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doping agents. In our laboratory a great deal of experimental work on this problem has been done. These experiments have shown that the combination of some diuretics with certain amphetamines could hinder the detection of the latter. In addition the loop-diuretics, furosemide and bumetanide decrease the excretion of phentermine, mephentermine, methylamphetamine and amphefamine with a factor of 5; the sensitivity of the method used was sufficient to demonstrate their presence even at the moment of maximum diuretic effect. The carbonic anhydrase inhibitor, acetazolamide, however, suppresses their excretion at the peak-moment of the urinary alkalinisation to such a degree that their detection becomes very difficult or impossible.

Thus it seems that loop-diuretics (furosemide, bumetanide) with their pure diluting effect do not have a significant influence on masking doping. Carbonic anhydrase inhibitors, on the contrary, do it very effectively not by means of dilution but by their alkalinising effect. This can be shown by the decreased excretion paralelled by urinary alkalinisation rather than urine dilution.

DETECTION AND IDENTIFICATION OF DOPING AGENTS

As I am not actually a race horse chemist, I will only refer to some general aspects of these techniques. Each analysis comprises three steps: extraction, detection and identification. The extraction procedure is in most cases identical for groups of related substances. For the detection and identification, however, a distinction must be made between endogenous and exogenous substances.

1. The detection of exogenous substances

1.1 Exogenous substances not related to therapy

This group comprises the sympathomimetic psychostimulants, the stimulant alkaloids and the stimulant narcotics (fentanyl, pentazocine, apomorphine and even etorphine). For these substances gaschromatographic or HPLC techniques are mostly used. Confirmation is obtained by means of GC-MS. It is sufficient to do only a qualitative analysis as these substances are exogenous to the body and their mere presence is sufficient to prove their use as doping agents.

1.2 Exogenous steroids

These include glucocorticoids (other than cortisone or hydrocortisone) and anabolic steroids (other than testosterone). We treat this group separately as their detection differs from the first group in the sense that concentrations are much lower in the urine (100-1 $\mu g.\ ml^{-1}$ in the former group versus picograms to nanograms ml^{-1} in this group).

Screening as well as identification must be carried out by means of the very sensitive technique of GC-MS after trimethylsilyl derivation, and must eventually be coupled to selected-ion-monitoring (SIM) techniques or RIA (radio immune assay) techniques.

1.3 Exogenous substances present in normal feed of horses

This problem is still one of the biggest to the doping analyst and up to the present no clear indications exist to aid the analyst in deciding to what limit their presence can be accepted as normal and when it should be regarded as due to intentional exogenous administration. Such substances include:

Salicylic acid: the administration of up to 65 mg kg^{-1} body mass produces urinary levels lower than those found after the ingestion of some types of grass and other feeds

- theobromine by ingesting cocoa-nut-shells present in the feed
- hordenine, an alkaloid with effects such as ephedrine or norephedrine, can be present in barley, malt and some Gramineae.

In race horses, the presence of caffeine in the urine is not a problem as it can not be considered to be present due to drinking several cups of coffee. In human athletes, on the other hand, it has been and still is a problem but recently a level of 15 $\mu g\ ml^{-1}$ urine was fixed as the upper acceptable limit. This means that for this group of substances in man a qualitative analysis must be accompanied by a quantitative one before a diagnosis of doping can be made.

1.4 Exogenous substances used for therapy

In this group, as for the foregoing, the debate still rages on as to how long before the start of a race they can be used. Two alternatives are proposed for the solution of this problem:

- either there is a total interdiction which means that no residues may be found in the urine and that a qualitative analysis is sufficient, or certain limits must be fixed below which a case is not considered as positive. Some are in favour of the latter as they contend that below certain concentrations, a therapeutic substance has no therapeutical effect and thus no doping effect. Urinary concentrations are not suited for these purposes as the volume and pH of urine fluctuate too much. Blood levels could be accepted as indications in the case of phenylbutazone, but only on condition that there is sufficient knowledge of the relationship between blood levels and therapeutic effect. A quantitative determination is thus also needed.

In either case, the problem for the practitioner still remains: How long before a race may he administer the substance. To answer this question, thorough knowledge of the kinetics is needed and adequate pharmacokinetic studies have to be done in order to obtain information on the plasma half life, the elimination half life, the volume of distribution, the total body clearance and the withdrawal period. At the same time the detection limit of the method used is essential.

2. The detection of endogenous substances

For cortisone or hydrocortisone no valid method is at present at the disposal of the laboratory. The question of testosterone is still under discussion: in human sport it has been solved by the acceptance of a ratio of 6 between the 17-β-testosterone and the 17-α-testosterone (or epitestosterone) concentrations. In female and gelding race horses the mere presence of testosterone confirms the administration of the drug. The presence of androstane 3-17-β-diol is also proof of this administration. An anabolic steroid that is strongly related to normal testosterone secretion is nortestosterone or nandrolone. Some years ago it was believed that the detection of 5α-oestrane-3,7β-diol was proof of the exogenous administration of nortestosterone. This is true for geldings and mares but recently it was found that this same diol is also an endogenous substance in the stallion. Thus in the stallion this method is not valid. However, at the last congress on doping in Hong Kong, it was demonstrated by Moss that the ratio of oestrane-3-17-β-diol and oestrene-3-17-β-diol can perhaps be of help in future for proving nortestosterone administration.

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THE CURRENT STATUS OF RESEARCH ON DISEASES OF WILDLIFE IN SOUTH AFRICA AND SOUTH WEST AFRICA/NAMIBIA+

ABSTRACT

This paper contains a review of the formal research being done on the diseases and parasitic infestations of wild animals by universities and the state as well as other institutions in this part of the African continent. It is clear that most information obtained in this research is of an epidemiological or ecological nature and that ungulates, which form the bulk of the game-farming industry, are receiving the most attention. An analysis of the 20 most important diseases, which cover 6 disciplines, revealed that most of the research is being done for the benefit of livestock rather than wildlife. Seventeen of these diseases can be regarded as indigenous and 3 as being exotic to this part of Africa. Arguments are provided in favour of the concept that our game is genetically endowed, by virtue of centuries of adaptation, to handle the indigenous diseases, if their resistance is not compromised. None of the indigenous diseases constitute a threat to game populations, and hence no research on them is justified from an economic point of view. What is, however, absolutely essential, is research on game management with particular reference to those ecological factors which enable game on game-fenced farms to lead the sort of lives to which they are adapted. If this approach is not followed, further destruction of the habitat and deterioration of genetic disease resistance seem inevitable.

Key words: Wildlife diseases, South West Africa, South Africa

Bigalke R.D. **The current status of research on diseases of wildlife in South Africa and South West Africa/Namibia.** *Journal of the South African Veterinary Association* (1989) 60 No. 1, 7-10 (En.) Veterinary Research Institute, P.O. Onderstepoort 0110, Republic of South Africa.

INTRODUCTION

I will start my talk by demarcating my territory in true wildlife fashion.

- I have concentrated on research being done at present or conducted during recent years.
- I am only speaking about research on wildlife diseases and parasites (henceforth referred to as "wildlife diseases") and have ignored work on embryo transfer and the anatomy and physiology of wild animals.
- I have only analysed research being done by what I regard as the large institutions, as listed in Table 1, and have assumed that institutions that did not respond to my request for information are not involved in wildlife research. Zoos were excluded from this survey.
- The main objective of my talk is to indicate what research is being done and what research I think should be done.

TEXT

Digest of research being done

Most of the research on wildlife diseases is being done in the Kruger National Park (KNP). I counted 33 research projects at present in progress of which only 7 are being led by KNP scientists. It is clear that there are a large number of guest scientists working on projects in cooperation with KNP and Veterinary Services scientists, who are located in the Park.

It is not surprising that the KNP is so popular.

- It is a treasure trove of jewels waiting to be discovered.
- It is virtually untouched by man.
- Its animals are subjected to diseases such as foot-and-mouth disease (FMD) and Corridor disease, which are of great economic importance to the livestock industry and consequently deserve a lot of attention.
- It is a most stimulating environment in which to do research.

I have listed the 9 main institutions doing research on wildlife diseases in Table 1. Between them they boast 76 research projects. The Tick Research Unit (TRU) has the largest number of projects in progress, but covers a narrow spectrum of disciplines. The Veterinary Research Institute, Onderstepoort runs a close second with 13 projects, and covers the widest spectrum of diseases. The Veterinary Faculty at the Medical University of Southern Africa is third, and so one can go on down the line.

It is clear that by far the majority of projects either have epidemiological studies as their main objectives or provide epidemiological information as a spin-off of other studies. This includes pathological and taxonomic studies which make a very valuable contribution to our knowledge of the epidemiology of wildlife diseases.

But, when one thinks of the embattled game-farming industry, and I will come back to this point, one wonders if the KNP deserves all this attention.

The species of wildlife involved

The species range of wildlife involved covers all mammal orders, and some other classes as well, i.e. from pisces to primates.

Antelope feature most often. Almost 42% of the projects study antelope, but it must be noted that I have lumped all the antelope together. It is nevertheless clear that antelope are at present receiving much attention in the field of research.

The large carnivores — ranging from jackal to larger species — and zebra enjoy a surprisingly high degree of attention in this field. Buffalo and wild pigs are important from a disease point of view and are also high on the list, as one would expect. The ungulates which include antelope, buffalo, wild pigs, hippos and rhinos, account for approximately 64% of the animals listed in Table 2.

The actual number of man hours spent on the various species has not been determined. I have in fact only totalled the names of animals which appear in project titles for the purpose of this analysis. One may well find, for instance, that more time goes into buffalo than into bird research, which sounds logical.

The impression has nonetheless been gained that the species priorities are more or less correct, from a disease importance point of view. The reason why I say this should become clearer at a later stage in this paper.

The scientific disciplines involved

More than a third of the projects (Table 3) deal with entomological research, of which more than half are TRU projects. Helminthology is next, followed by virology.

Once again the priorities are correct, although much of the interest in helminthology is what might be termed "curiosity research". The next table (Table 4) indicates that some of the most important diseases have a viral aetiology, suggesting that virology should probably be first rather than third in line.

Important diseases being studied

In table 4, I have listed what I regard as the 20 most important diseases of wildlife, from an economic point of view, on which research is currently being done. The spectrum covers 6 disciplines. I have also tried to identify whether the main beneficiary of this research is livestock or wildlife. If any doubt existed in my mind, a zero mark was allocated. Despite the relative lack of information about wildlife diseases, I think my deductions are probably fairly close to the mark.

It is clear from Table 4 that only 7 of the 20 diseases (35%) are known to cause ill

+ Paper delivered at Wildlife Diseases Symposium held at Skukuza, Kruger National Park from 26-27 August 1987 under the auspices of the Pathology and Wildlife Groups of the South African Veterinary Association.

Institution	No. of Projects	Diseases/Infection	Epidemiology	Immuno-prophylaxis	Cure	Aetiology	Pathogenesis
National Parks Board	7	anthrax	+	+			
		blowflies	+				
		ectoparasites	+				
		biting midges	+				
		rinderpest	+				
Natal Parks Board	7	ectoparasites	+				
		internal parasites	+			+	+
		blindness				+	
		various (pathology)	+			+	+
Faculty of Veterinary Science, University of Pretoria	7	strongylosis	+				
		internal parasites	+				
		haemoprotozoa	+				
		paramyxo-virus				+	
		neonatal disease				+	
		unhealthy food	+			+	
Faculty of Veterinary Science, Medical University of Southern Africa	10	ectoparasites	+				
		internal parasites	+				
		reproduction	+			+	
		pathology	+			+	+
Tick Research Unit, Rhodes University	15	ticks (& tick-borne dis.)	+				
		internal parasites	+				
Veterinary Services SWA/Namibia	6	uitpeuloog	+				+
		cytauxzoonosis	+				+
		plant poisoning	+				
		rabies	+	+			
Nature Conservation SWA/Namibia	6	anthrax	+				
		mange	+				
		alopecia	+			+	
		rabies	+	+			
Veterinary Services RSA	1	tsetse flies	+				
Onderstepoort Veterinary Research Institute	13	**Culicoides**	+				
		Karoo tick paralysis	+				
		ticks	+				
		internal parasites	+				
		heartwater	+				
		theileriosis	+				
		besnoitiosis	+	+			
		turkey malaria	+				
		foot-and-mouth disease	+				
		snotsiekte	+				
		African swine fever	+				
		equine influenza	+				
		fish diseases	+		+		
Other	4	internal parasites	+				
		schistosomes	+				
		sarcocystosis	+				
		haemorrhagic fevers	+				
		leukaemia	+				

health in wildlife. Five of them also cause disease in livestock. Only 2, therefore, namely cytauxzoonosis and sarcoptic mange of jackals, are studied solely for the benefit of wildlife. Conversely, 15 of the 20 diseases (75%) are important causes of ill health in livestock and 10 (50%) are studied solely for their benefit. I don't think there can be any doubt that most of the current research is being done for the benefit of livestock.

The big killer diseases of wildlife are rinderpest and anthrax. We all know what havoc rinderpest caused to the buffalo population and other big game at the turn of the century. We may also have heard about the big buffalo losses which occurred recently in Tanzania. Almost 60% of animal deaths in the Etosha area are caused by anthrax, and we know that this disease has received much research attention in the KNP. Rabies can play an important role in wildlife health, for example in kudu in SWA/Namibia. Gifblaar can kill game, but only under exceptional circumstances. Foot-and-mouth disease and equine influenza can hardly be regarded as killer diseases amongst wildlife. Cytauxzoonosis regularly kills tsessebe calves in certain Transvaal reserves. However, many more deaths could be expected amongst tsessebe, roan, sable, giraffe, kudu and duiker if cytauxzoonosis were a primary killer.

Resistance and susceptibility of wildlife to diseases

In Table 5 I have divided the 20 most important diseases of wildlife into indigenous and exotic diseases.

a. Indigenous diseases are those diseases which, I think, have been here from time immemorial, i.e. the wild animals have been exposed to them during their evolutionary development (in a specific ecosystem). In my opinion it can be stated with fair confidence, that with the exception of rabies, wildlife populations can normally handle these diseases successfully.

Let me name a typical example: It is common knowledge that wild animals

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Table 2: **Animal groups featuring in projects**

Animal	No. of projects	%
antelope	39	41,9
large carnivores	9	9,7
zebra	8	8,6
birds	7	7,5
buffalo	5	5,4
wild pigs	5	5,4
hares	4	4,3
rodents	4	4,3
primates	2	2,2
fish	2	2,2
bats	2	2,2
hippos	1	1,1
rhinos	1	1,1
dassies	1	1,1
dolphins	1	1,1
tortoises	1	1,1
snakes	1	1,1

can survive quite happily in tsetse-infested areas where domesticated animals succumb to trypanosomiasis. We all know why. We refer to the phenomenon in general terms as survival of the fittest, natural selection or adaptation.

Adaptation would also explain the apparent resistance of wildebeest to snotsiekte, buffalo to FMD and Corridor disease, warthog to African swine fever (ASF), antelope to heartwater, zebra to strongylosis, antelope to ecto- and endoparasitic infestations, and eland to poisoning with gifblaar (*Dichapetalum cymosum*). It is not known how this resistance operates, but it is reasonable to assume that a more efficient immune response is involved in the phenomenon of adaptation to infectious and parasitic diseases. There is both circumstantial and more concrete evidence that it has a genetic basis.

Let us consider more specifically the adaptation of ungulates, as they are game-ranched and will form the backbone of the game industry when it becomes a stable industry.

Please remember that:

* Adaptation developed over many centuries, not overnight.
* Adaptation developed in the complete absence of acaricides, worm remedies, antibiotics, other pharmaceuticals and vaccines.
* Migration forms part of adaptation, i.e. there were no game fences around in those days.
* Migration means unhindered movement, to a greater or lesser extent, which means a low stocking rate, considerably lower than the stocking rates applicable to cattle and sheep, for example.

There is also some evidence that our wildlife cannot handle indigenous diseases if their resistance is compromised by stress. We have heard today of dominant impala rams carrying higher tick burdens; a blue wildebeest with a broken leg carrying more larval and nymphal ticks than others in the herd and even fairly large numbers of adult ticks, which is most unusual in this species; massive worm infestations are seen in impala that are food-stressed during droughts, and coccidiosis is a regular and severe problem in recently-captured impala.

Although we could do with some more evidence, I think it can be stated, with few exceptions, that if indigenous diseases cause ill health, they are either secondary to other debilitating conditions or the animals concerned do not belong in that particular ecosystem. To recap, our wildlife can handle indigenous diseases if they are allowed to live in the manner, to which they are adapted.

When one speaks of adaptation to disease, it is of course important to think in terms of a population of animals, the bulk of which is resistant. This implies that some individuals are less resistant than others.

b. Exotic diseases are those diseases to which our wildlife were not exposed during their evolution. There are only 2 of the 20 listed in Table 5 that are definitely exotic, namely rinderpest and equine influenza.

Is anthrax an exotic disease? Evidence indicates that it was brought into the KNP region by man approximately 200-250 years ago. This is much too short a period for adaptation to have taken place and would explain why many wildlife species in the KNP, Etosha and elsewhere are highly susceptible to anthrax.

I regard rabies, particularly the type found amongst wildlife, as being indigenous, but the incidence has probably been too sporadic to exert selection pressure for resistance.

Justifications for research on wildlife diseases

The question arises whether we should do more research on wildlife diseases than is already being done.

If the aim is to control diseases, there are in my opinion 3 reasons why more money should be spent on research into wildlife diseases!

1. **If the diseases pose a threat to wildlife populations**
   I have already spoken about the havoc wrought by anthrax and rinderpest.
2. **If the diseases pose a threat to the agricultural economy**
   FMD is not only a very serious threat to our agricultural export trade, but also threatens the existence of the KNP itself. As long as FMD is controlled as effectively as is being done at present, all will be well. But if the disease should spread beyond its accepted environs, the pressures will build up again. FMD also denies realisation of the full economic potential of large tracts of cattle and game-farming country along the borders of the KNP.

   Corridor disease is not a threat to our export trade, but it resembles FMD in many other respects, and "massacres" cattle to boot.

   As far as snotsiekte (malignant catarrhal fever) is concerned, we have allowed this wildebeest "terrorist, armed with lethal weapons" to penetrate right into our midst, and there is no solution in sight.

   ASF is potential dynamite, but fortunately at present there is apparently an epidemiological barrier in operation.

   Uitpeuloog (gedoelstiasis) has much of the same potential for trouble as snotsiekte, but for reasons which are not quite clear the condition does not appear to be as prevalent.

Table 3: **Number of projects per discipline**

Discipline	No. of projects	%
Entomology	22	29
Helminthology	14	18,4
Virology	14	18,4
Entomology & Helminthology	7	9,2
Protozoology	7	9,2
Pathology	6	8
Bacteriology	3	4
Reproduction	1	1,3
Toxicology	1	1,3
Veterinary Public Health	1	1,3
	76	

Table 4: **Important diseases being studied**

Disease/infestation	Animal species	Possible beneficiaries Stock	Wildlife
Anthrax	several	+	+
Heartwater	guinea fowl; tortoise	+	o
Karoo tick paralysis	various spp.	+	o
Sarcoptic mange	jackal	o	+
Tick infestation	several	+	o
Uitpeuloog	blue wildebeest	+	o
Schistosomiasis	hippopotamus	o	o
Strongylosis	zebra	+	o
Worm infestation	several	o	o
Besnoitiosis	blue wildebeest	+	o
Corridor disease	buffalo	+	o
Cytauxzoonosis	several antelope spp.	o	+
Sarcocystosis	several	o	o
African swine fever	warthog	+	o
Equine influenza	zebra	+	+
Foot-and-mouth disease	buffalo and other game	+	+
Rabies	kudu	+	+
Rinderpest	buffalo and other game	+	+
Snotsiekte	blue and black wildebeest	+	o
Gifblaar	eland	+	o

3. If the diseases pose a threat to the health of man

Rabies is the obvious example, and we know that it can also threaten big kudu-

Table 5: **Diseases of wildlife**

Indigenous	Exotic
Heartwater	Anthrax
Karoo tick paralysis	
Sarcoptic mange	
Tick infestation	
Uitpeuloog	
Schistosomiasis	
Strongylosis	
Worm infestation	
Besnoitiosis	
Cytauxzoonosis	
Sarcocystosis	
Corridor disease	
African swine fever	Equine influenza
Foot-and-mouth disease	Rinderpest
Rabies	
Snotsiekte	
Gifblaar	

populations, and also livestock. The rabies problem in kudu, however, has apparently been associated with an overpopulation of that antelope and has proved to be self-limiting.

There are therefore only 8 of the 20 wildlife diseases, of which 5 are indigenous, which justify large research inputs. Only 2 of the 8 diseases, namely anthrax and rinderpest, pose a threat to wildlife populations. This means that only 2 of the 20 important diseases being studied, justify large research inputs, from the wildlife point of view.

Wildlife management considerations

This reasoning must not be interpreted as meaning that no research on wildlife pertaining to their indigenous diseases is necessary. However, a radical change in approach is, absolutely essential. The reason why indigenous diseases do not justify extensive research inputs per se is because wildlife are able to handle these diseases if they are allowed to lead the kind of lives to which they are adapted. This means that they can manage quite well without dips, worm remedies, antibiotics, other drugs, vaccines and additional feeding, provided that their genetically determined defence mechanisms are not compromised. The ecological factors that enable game to lead the lives to which they are adapted, need to be identified.

Since true migration is not possible on most game-fenced farms, the condition of the habitat becomes a crucial factor. A suitable habitat can only be provided if the farms are managed correctly. The fact that approximately 60% of game farms are currently overgrazed is a clear indication that the fundamental information required for the proper management of game farms or mixed game and stock farms is lacking. This information has a direct bearing on indigenous diseases of game, as has already been pointed out.

The following ecological research, with special reference to long-term management of game-fenced farms, is very urgently required:

- A shift in emphasis from diseases of game per se to ecological research aimed at the long-term management of game-fenced farms.
- A determination of the long-term carrying capacity of game-fenced farms for various animal species in various ecological regions.
- The development of long-term guidelines to determine which proportion of the population of each species present on such farms to harvest annually.
- The development of guidelines to evaluate the condition of the veld as an aid to management of game-fenced farms.
- The determination of minimum sizes for game-fenced farms in various regions.
- Integration of the above information into a management system that will have as its primary objective, conservation of the habitat rather than of the wildlife.

Our motto should become: **If we conserve the habitat, the wildlife will look after itself.**

There are a number of veterinarians qualified to do this kind of work. It is, however, primarily the task of the ecologist, grassland expert and animal scientist. The role of the veterinary scientist could well be to become a member of such a research team. I see him using his physiological, pathological and parasitological knowledge, and perhaps above all his sophisticated array of clinical pathological tests to augment the production-orientated studies of the animal scientist to help determine whether game-ranched animals live in harmony with the environment or are under stress. Tick and worm burdens could for instance prove to be valuable stress indices.

CONCLUSION

As far as the future is concerned, I think some rationalisation of research is necessary. A certain amount of curiosity research is essential. Let us, however, who have the health of our wildlife at heart, not become blinded by the disease aspect. I appeal to you not to make the fatal mistake of trying to turn wild animals into domesticated animals. If you do, you will not only be contributing to further destruction of the habitat, but also be counteracting natural selection, and gradual genetic deterioration will be the result. Fortunately, evolutionary adaptation stretching over millions of years, cannot be reversed overnight.

It has taken us decades to realise that we cannot do without genetic resistance in domestic stock. Let us use this wonderful gift of nature, which dates back to antiquity, correctly from the outset.

R.D. Bigalke, Veterinary Research Institute, Onderstepoort.

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SERUM DIGOXIN CONCENTRATIONS IN CANINE CONGESTIVE HEART FAILURE

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ABSTRACT

Digoxin was administered to dogs (n = 10) in congestive heart failure, at an oral dosage rate of 0.01 mg kg^{-1} lean body mass twice daily. Lean body mass was determined by reducing gross mass by the estimated degree of ascites and body fat. The dose was further adjusted for factors such as renal and hepatic function, the bioavailability of different formulations, and the size of the patient. Trough and peak serum digoxin concentrations were determined after 10 days of digitalisation, or when signs of toxicity became apparent.

Serum digoxin concentrations in 6 of the 10 dogs were found to be partially or completely in the toxic or subtherapeutic range. This indicates that an oral digoxin dosage rate of 0.01 mg kg^{-1} lean body mass administered twice daily, even when adjusted appropriately for factors that affect digoxin pharmacokinetics, provides no more than a rough approximation of the precise dose required to provide serum digoxin concentrations within the therapeutic range. The observations also lend support to a recent recommendation that the digoxin dosage rate should be based on body surface area, although even when administered on this basis, serum digoxin concentrations outside of the therapeutic range could be anticipated.

Key words: Oral digoxin dosage rate, dogs, serum digoxin concentration determinations

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INTRODUCTION

Since William Withering published his classic monograph "An account of the Foxglove and some practical remarks on dropsy and other diseases" in 1785, the digitalis glycosides have been used extensively for the treatment of cardiac disorders in animals and man[1]. With few exceptions, this group of drugs is indicated as primary or adjunctive therapy in all cases of congestive heart failure, and for the treatment of supraventricular tachyarrythmias[15].

The 2 oral digitalis glycosides used most commonly in veterinary medicine are digoxin and digitoxin. Digoxin is the preferred drug because it has a greater negative chronotropic effect and a longer half-life than digitoxin. Digoxin also produces greater gastroenteric side-effects with overdose than digitoxin, which portend the more important cardiotoxic effects[12]. The pharmacokinetics, bioavailability and dosage regimen of digoxin has been thoroughly investigated[3 4], while no such studies have been conducted for digitoxin.

The cardiac glycosides have an extremely narrow therapeutic index. Reported therapeutic ranges for digoxin include serum concentrations of 0,8 - 2,0 $\mu g\ \ell^{-1}$ and 1,0 - 2,4 $\mu g\ \ell^{-1}$ [12]. Serum digoxin concentrations greater than 2,5 $\mu g\ \ell^{-1}$ are considered to fall within the toxic range[8]. Signs associated with toxicity include depression, anorexia, vomition, diarrhoea, a variety of cardiac arrythmias, and death[11 12]. Subacute digoxin intoxication can result in degenerative lesions in the myocardium and the kidneys[16]. It is therefore fundamentally important for the clinician to ensure that digoxin is administered at an appropriate dose to provide stable serum concentrations within the therapeutic range.

A variety of dosage regimens for digoxin have been published. Most of these were developed empirically, although one regimen is based on a thorough pharmacokinetic study[3]. There is general agreement that loading doses of digoxin should be avoided, except in emergency situations. Recommended oral maintenance[3 5] dosage rates range from 0,020 mg $kg^{-1}\ 24h^{-1}$ to 0,023 mg $kg^{-1}\ 24h^{-1}$. These dosages should be divided and given every 12 h, and should result in equilibrated serum digoxin concentrations in the therapeutic range after approximately 7 d[11].

The recommended dosage rates for digoxin are based on lean body mass. Digoxin is poorly lipid soluble, and it does not distribute into ascitic fluid[4 12]. Body mass should be reduced by 10–, 20–, 30– and 40% for mild, moderate, severe and very severe ascites, respectively[4]. Similar figures are not available for obesity, but body fat is estimated to comprise 15% of total body mass in normal individuals, and a substantial correction is required when estimating lean mass in an obese patient[11 12].

Digoxin is excreted via the kidneys primarily in the unchanged form. Only about 15% of digoxin is biotransformed in the liver[11]. If renal function is impaired, the digoxin dose should be markedly reduced. It has been recommended that the dose of digoxin should be decreased by 50% for every 18 mmol ℓ^{-1} increase in blood urea[11 14]. Serum creatinine, however, has been shown to more accurately reflect glomerular filtration and is also less affected by extrarenal factors[6]. Similarly, a small decrease in the digoxin dose should accompany clinical or biochemical indications of hepatic impairment[11].

Other factors that affect the dose of digoxin are the bioavailability of different formulations[3], the size of the patient[11 12] and concurrent therapy with certain drugs[11]. The elixir has a greater oral bioavailability than the tablets, and the dose of digoxin should be reduced by approximately 10% when the elixir is used[12]. Larger dogs require lower dosage rates on a per kilogram basis than smaller dogs[11 12], although specific recommendations in this regard are not available. Lower doses of digoxin should be used when drugs such as quinidine and verapamil, which affect the volume of distribution and excretion of digoxin, are administered concurrently[1 10]. Simultaneous oral administration of digoxin with medications such as antacids, kaolin and pectin, which inhibit the absorption of the digitalis glycoside, should be avoided[11].

Certain electrolyte disturbances may potentiate digitalis arrythmogenicity. Low serum potassium is particularly important in this regard, because it increases digitalis-tissue receptor binding[1]. Hypokalaemia, if present, should therefore be corrected.

All dogs to which digoxin is administered at the Onderstepoort Veterinary Teaching Hospital, University of Pretoria, are digitalised according to the above general recommendations. Serum digoxin concentrations have been determined in some patients to evaluate the adequacy of this digitalisation procedure, and to consider the value of determining serum digoxin concentrations in clinical cases.

MATERIAL AND METHODS

Ten dogs suffering from congestive heart failure, either due to mitral valvular incuffiency (n = 3), idiopathic congestive cardiomyopathy (n = 6) or ventricular septal defect (n = 1) were selected for serum digoxin concentration determinations. The diagnoses were made on the basis of signalment, history, physical examination, electrocardiography, radiology and analysis of ascitic fluid, where applicable. The ages of the dogs ranged from 2 to 14 years, and gross body mass ranged from 8 to 48 kg. Routine

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haematology, blood smear examination, urinalysis and faecal flotation were performed in each case.

The lean body mass of each dog was determined by decreasing the gross mass by the estimated degree of ascites and body fat. With the exception of one case, a biochemical profile comprised of serum creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP) and potassium was performed for each dog.

An oral digoxin (Lanoxin, Wellcome) dosage rate of 0,01 mg kg^{-1} lean body mass administered twice daily was selected. A lower dosage rate was used in larger dogs than in smaller dogs. The size of this adjustment was made at the discretion of the consulting clinician. The dose was reduced by 50% for every doubling of the serum creatinine level. The dose was further reduced by 10% where elevations in ALT and/or ALP indicated impaired hepatic function. A 10% lower dose was used where the elixir was administered. No drugs that are known to affect digoxin bioavailability, volume of distribution or excretion were administered concurrently.

After 10 d of digitalisation, or if signs of toxicity occurred, a serum sample was obtained 12h after the previous digoxin administration (the trough serum digoxin sample). Digoxin was administered immediately after sampling, at the calculated dose, and a serum sample was obtained 45 min after administration of elixir or 2 h after administration of tablets (the peak serum digoxin sample). Serum digoxin concentrations were determined by radio-immunoassay (Digoxin Solid Phase Component System, Becton-Dickenson, New York) within 24 h. In all cases where serum digoxin concentrations were found to be outside of the therapeutic range, appropriate dose adjustments were made to establish therapeutic concentrations.

RESULTS

The various parameters considered in the adjustment of the digoxin doses administered to the 10 dogs are given in Table 1. The serum potassium concentrations were within the normal range (3,6 - 5,1 mmol ℓ^{-1}) for all the dogs. The estimated lean body masses, prescribed digoxin doses, serum digoxin concentrations and clinical signs of toxicity are listed in Table 2.

Three dogs were found to have serum digoxin concentrations either partially or completely in the toxic range (Dog 1,4 and 7). Two of the 3 dogs showed signs consistent with digoxin toxicity.

Four dogs had serum digoxin concentrations in the therapeutic range (Dog 2, 8, 9 and 10). In 2 of these cases (Dog 8 and 9), digoxin dosages substantially lower than 0,01 mg kg^{-1} twice daily were prescribed, especially in the case of Dog 9.

Three dogs had serum digoxin concentrations that were partially within the subtherapeutic range (Dog 3, 5 and 6). A slightly low digoxin dose was prescribed for Dog 5, but the doses administered to Dog 3 and 6 were considered appropriate.

DISCUSSION

The results of this study indicate that a digoxin dosage rate of 0,01 mg kg^{-1} lean body mass, corrected for various factors that affect digoxin pharmacokinetics and administered twice daily, provides no more than a rough approximation of the precise dose required to obtain serum digoxin concentrations within the therapeutic range in the dog. This is consistent with a survey involving 79 dogs on digoxin therapy, where 25% of the dogs had serum digoxin concentrations in the toxic range, and 24% were underdigitalised[11]. Similar human studies have shown that between 23% and 29% of patients taking digitalis glycoside preparations show signs of toxicity[2]. These findings are cause for considerable concern. Although subtherapeutic serum digoxin levels do not pose any danger to the patient, they obviously result in suboptimal therapeutic effects. The consequences of digoxin intoxication however, are extremely serious, and may be fatal in some cases.

An oral digoxin dosage rate based on body surface area (i.e. 0,22 mg/m^2 administered twice daily) has recently been recommended. Body surface area is used extensively for drugs with narrow therapeutic indices, such as cancer chemotherapeutic agents, as it predicts volume of distribution of the drug more precisely than body mass. Since larger dogs have a lower body surface area to body mass ratio, they would require less digoxin on a per kilogram basis than smaller dogs, to obtain the same serum digoxin concentrations[8]. In a reported study of 9 dogs with idiopathic congestive cardiomyopathy, digoxin was administered at a dosage rate of 0,22 mg/m^2 twice daily, and all of the dogs were found to have serum digoxin concentrations in the therapeutic range after

Table 1: Various parameters considered in the adjustment of the dose of digoxin in 10 dogs in congestive heart failure

No	Signalment	Diagnosis	Estimated ascites (%)	Estimated body fat (%)	Creatinine (μmol ℓ^{-1}) N = < 133	ALT (U ℓ^{-1}) N = < 40	ALP (U ℓ^{-1}) N = < 190
1	Chow, female 7 years, 24 kg	VSD	30	10	117,8	16	226
2	Labrador, male 9 years, 24 kg	ICC	0	15	100,0	35	154
3	Terrier cross, female 14 years, 8 kg	MVI	0	25	ND	ND	ND
4	Bouvier, male 7 years, 28 kg	ICC	0	15	111,0	21	22
5	Terrier cross, female 10 years, 8 kg	MVI	0	15	43,1	5	86
6	Standard Poodle, male 8 years, 32 kg	ICC	0	15	98,5	131	68
7	Bouvier, male 10 years, 48 kg	ICC	20	10	176,0	56	24
8	Collie cross, male 7 years, 20 kg	MVI	0	10	108,1	10	61
9	Doberman, female 5 years, 42 kg	ICC	0	20	106,6	13	62
10	Doberman cross, female 3 years, 32 kg	ICC	0	15	124,6	10	126

VSD = Ventricular septal defect
ICC = Idiopathic congestive cardiomyopathy
MVI = Mitral valvular insufficiency
ND = Not done
ALT = Alanine transaminase
ALP = Alkaline phosphatase
N = Normal

Table 2: Estimated lean body masses, oral digoxin doses administered, serum digoxin concentrations and clinical signs of toxicity in 10 dogs in congestive heart failure. Body surface area's and proposed oral digoxin doses based on body surface area are included for comparative purposes.

No.	Breed	Estimated lean body mass (kg)	Oral digoxin dose administered twice daily (mg)	Trough serum digoxin concentration ($\mu g\ \ell^{-1}$)	Peak serum digoxin concentration ($\mu g\ \ell^{-1}$)	Clinical signs of toxicity	Body surface area** (m^2)	Proposed oral digoxin dose for twice daily administration based on body surface area*** (mg)
1	Chow	14,4	0,1225 (elixir)	3,3	4,6	Depression; second degree heart block	0,62	0,1364
2	Labrador	20,4	0,1875 (tabs)	1,5	2,2	-	0,78	0,1716
3	Terrier cross	6,0	0,06 (elixir)	0,6	1,4	-	0,34	0,0748
4	Bouvier	23,8	0,1875 (tabs)	3,7	5,5	Depression; anorexia; vomition	0,86	0,1892
5	Terrier cross	6,8	0,05 (elixir)	<0,5	1,6	-	0,37	0,0814
6	Standard Poodle	27,8	0,25 (tabs)	0,5	1,6	-	0,96	0,2112
7	Bouvier	33,6	0,25 (tabs)	1,9	3,1	-	1,09	0,2398
8	Collie cross	18,0	0,125 (tabs)	0,9	1,6	-	0,72	0,1584
9	Doberman	33,6	0,125 (tabs)	1,4	2,2	-	1,09	0,2398
10	Doberman cross	27,2	0,25 (tabs)	0,9	2,1	-	0,95	0,2090

* The therapeutic range for serum dogoxin is 0,8 to 2,4 $\mu g\ \ell^{-1}$, and concentrations greater than 2,5 $\mu g\ \ell^{-1}$ are considered to fall within the toxic range.

* Body surface area is determined by the following formula[7]:

$$BSA = \frac{K \times W^{2/3}}{10^4}$$

Where BSA = Body surface area in m^2
W = Mass in g, and
K = 10,1

*** The digoxin dose is based on a dosage recommendation of 0,22 mg per square metre [8].

5 d[9]. Body surface area may be determined by using a mathematical formula[7] (Table 2), and conversion tables of mass to body surface area are available in various veterinary texts[7 13]. Adjustments of the digoxin dose for factors known to affect digoxin pharmacokinetics, should obviously still be made.

The observations made in the cases comprising the present study, support the concept of digoxin administration based on body surface area. The 3 dogs found to have digoxin concentrations in the toxic range (Dog 1, 4 and 7) were all fairly large, with gross masses exceeding 24 kg. In 2 large dogs (Dog 8 and 9) where digoxin concentrations were found to be in the therapeutic range, substantial dose reductions for the size of the dogs were fortuitously made: Oral digoxin administration based on the proposed dosage rate of 0,22 mg/m^2 twice daily, however, would not consistently result in serum digoxin concentrations in the therapeutic range. In Dog 1 and 4, where both trough and peak serum digoxin concentrations were obtained in the toxic range, slightly higher digoxin doses would have been administered if a dosage rate of 0,22 mg/m^2, based on a conversion from lean body mass, had been selected. In Dog 7, where the peak serum digoxin concentration was found to be in the toxic range, only a marginally lower digoxin dose would have been administered. In Dog 6, where serum digoxin concentrations were obtained in the partially subtherapeutic range, approximately 20% less digoxin would have been administered if a dosage rate of 0,22 mg/m^2 had been used (Table 2).

Digoxin toxicity is a commonly recognised problem in human cardiac patients[2]. Consequently, reasonably inexpensive serum digoxin concentration determinations have become readily available through most clinical pathologists. Various protocols for determining serum digoxin concentrations have been recommended, including single determinations at 8 to 10 h after digoxin administration. The dose is considered appropriate if the serum digoxin concentration in this sample falls within the therapeutic range[12]. In the present study, peutic range[12]. In the present study, trough and peak concentrations were determined after at least 7 d, when serum digoxin concentrations would have equilibrated, or if signs of toxicity occurred. Trough concentrations were obtained 12 h after the previous digoxin administration, immediately prior to dosing. Peak serum concentrations have been shown to occur at 45 min or 2 h after administration of the elixir or tablets, respectively[3 11].

Trough and peak serum digoxin concentration determinations were extremely useful in the management of the cases in this study. They provided precise confirmation of serum concentrations in the subtherapeutic, therapeutic or toxic ranges, and an objective basis for dose adjustment where necessary. In selected heart failure cases, where response to digoxin therapy appears inadequate, or where clinical signs suggestive of toxicity arise, determination of serum digoxin concentrations would provide valuable information for the further management of the patient.

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INTERAKSIES TUSSEN VEEARTS, KLIËNT EN PASIËNT

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ABSTRACT

From the literature it has been established that pets can fulfil specific functions with regard to their owners. The purpose of this investigation is to highlight the interaction which takes place between pet owners and their pets as observed during consultations with veterinarians. The emphasis is however on the meaning of the emotional content of the interaction rather than on the actual functioning of the interaction. The interactions were monitored by means of natural observation, information gained through the completion of questionnaires, information about the client obtained from the veterinarian and content analysis of taped conversations which took place during consultations. The results indicate that the interaction between a pet owner and a pet is one of need fulfilment.

Content analysis indicated that the owners attempt to fulfil certain needs through their pets, or conversely attempt to project their own emotions onto their pets. Loneliness and aggression are typical examples of this. A veterinarian who is able to identify his client's emotional needs, can alleviate his problems by giving unconditional support, referring the client with serious emotional problems to a psychologist or consulting a psychologist on behalf of the client.

Key words: Veterinary clients, emotional needs, veterinarian, psychologist

Odendaal J.S.J; Scheepers E.; Nel R. **Interactions between the veterinarian, client and patient.** *Journal of the South African Veterinary Association* (1989) 60 No. 1, 15-19 (Afr) Department of Zootechnology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INLEIDING

Daar word vermoed dat mens-tot-geselskapsdier-interaksies ongeveer 12 000 jaar gelede ontstaan het[5]. Dit word vandag aanvaar dat geselskapsdiere 'n rol kan speel in die bevrediging van 'n mens se emosionele behoeftes[1,6,8]. Die interaksie tussen mens en geselskapsdier waarby die sosiale ruimtes van beide beïnvloed word, neem in die moderne samelewing toe in kompleksiteit en intensiteit.

Leigh[9] wys daarop dat die verskillende soorte verhoudings tussen die eienaar en die geselskapsdier grootliks deur die eienaar bepaal word. Die spesifieke waarde wat 'n persoon aan sy geselskapsdier toeskryf word 'n spieël van dit wat hy wil wees en kwaliteite waaroor hy nie beskik nie, word in die dier geëksternaliseer. 'n Voorbeeld hiervan is moontlik die onselfgeldende man wat 'n grootras hond aanhou. Dit staan bekend as andromorfisme. Verder kan die eienaar haar- of homself as 'n redder van diere sien en sulke eienaars is dikwels by die welsyn van diere betrokke. Oormatige klem op dierewelsyn, waar die diere byvoorbeeld as kindersubstitute aangewend word, word 'n wyse waarop die persoon haar of sy emosionele behoeftes kan bevredig. Dit staan soms bekend as die najaag van sogenaamde reddingsfantasieë. Nog 'n voorbeeld van hoe 'n eienaar sy geselskapsdier kan gebruik om sy ele behoeftes te bevredig, is die narcistiese (selfliefde) rol wat die dier in die eienaar se lewe speel, om die eienaar se selfbeeld en persoonlikheid te bevorder. Vir Yoxall[12] behoort kennis oor diere en menslike gedrag vir die veearts ewe belangrik te wees. Die redes hiervoor is die toenemende rol wat geselskapsdiere in die moderne samelewing speel, sowel as die voorkoms van 'n groot verskeidenheid mens-dier-interaksies. Yoxall[12] tipeer die interaksies van veeartskliënte in die veeartskonsultasie-kamer as volg: (i) kliënte wat die veearts vrees; (ii) kliënte wat verskeie geselskapsdiere aanhou en (iii) neurotiese kliënte.

Odendaal[11] meen dat daar twee hoof kategorieë veterinêre konsultasies onderskei kan word. Hierdie tipering van konsultasies is gedoen na aanleiding van die kliënt se motivering om die veearts te besoek. Die eerste kategorie bestaan uit kliënte wat die veearts besoek omdat hulle diere werklik siek of beseer is. Die tweede kategorie bestaan uit kliënte wat die veearts besoek om hulle ele onderliggende emosionele behoeftes te bevredig. Die dier word in laasgenoemde geval gebruik as 'n tussenganger. Die kliënt wat die veearts dikwels weens emosionele redes besoek, staan onder veeartse bekend as "neurotiese" kliënte.

Hierdie kliënte moet nogtans as 'n integrale deel van die veeartspraktyk beskou word. In die lig van die interaksies tussen eienaar en geselskapsdier is dit van pas dat die nut van geselskapsdiere as terapeutiese ondersteuning ook in ag geneem word. Odendaal[11] beklemtoon dat die veearts noodwendig betrokke is by sulke interaksies tussen kliënt en geselskapsdier. Die rol van die veearts sal in hierdie verband belangriker word namate geselskapsdiere meer om emosionele redes aangehou word. 'n Voorbeeld hiervan is waar die geselskapsdier aangewend word in 'n spanningsontladinghoedanigheid deurdat die eienaar byvoorbeeld met die hond gaan stap of met hom speel. Die veearts se betrokkenheid by die menslike aspek van sy praktyk is 'n verantwoordelikheid waarvan hy nie kan ontkom nie.

Uit die literatuur is dit duidelik dat verskeie funksies wat deur geselskapsdiere vir hulle eienaars vervul word beskryf is. Daar word egter selde betekenis aan hierdie funksies toegeskryf. Die doel van hierdie studie is om die interaksies in die konsultasiekamer van die veearts te beskryf. Uit so 'n beskrywing kan daar groter begrip bewerkstelllig word ten opsigte van die funksies wat geselskapsdiere vir hulle eienaars vervul. Dit sluit dan ook 'n beskrywing in van hoe kliënte die konsultasie by 'n veearts ten opsigte van hulle ele emosionele behoeftes, benut.

MATERIAAL EN METODE

Nege voorstedelike veeartspraktyke is genader om in die ondersoek deel te neem. Twee praktyke was bereid om hulle fasiliteite beskikbaar te stel. Die veeartse is ten volle ingelig oor die aard van die ondersoek.

Die ondersoek het bestaan uit onderhoude met vier kliënte wat die veearts gekonsulteer het. Die kliënte wat eerste opgedaag het vir 'n spesifieke konsultasiesessie op 'n gegewe dag by die onderskeie veeartspraktyke, is geselekteer as proefpersone.

Die ondersoek is as volg uitgevoer:

(i) Mondelinge vrae is aan die kliënte voorgelê aan die hand van 'n vraelys, voor konsultasie in die wagkamer.

(ii) Waarneming is gedoen van die kliënte se algemene gedrag in die wagkamer, voor konsultasie.

(iii) Waarneming van kliënte is gedoen tydens konsultasie met betrekking tot nieverbale gedrag.

(iv) 'n Bandopname van die konsultasiesessie is gemaak met betrekking tot die verbale gedrag.

(v) 'n Onderhoud is met die veearts gevoer ten opsigte van inligting oor die kliënt, sowel as algemene kliniese inligting ten opsigte van die geselskapdier.

Die vraelys is saamgestel met die oog op die evaluering van die kliënte se verhouding met hulle geselskapsdiere, sowel as om die funksie wat die diere vervul vas te stel. Die vraelys is gebruik om verdere gedetaileerde inligting te bekom ten opsigte van die intensiteit van die eienaar se betrokkenheid by die geselskapsdier. Hier is daar spesifiek gekonsentreer op die volgende:

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Ontvang: Junie 1988 Aanvaar: September 1988

(i) Watter aktiwiteite saam met die dier gedoen word. (Byvoorbeeld die neem vir oefening, besoeke aan die veearts, tipe voedsel, slaapplek, die bad van die dier en of die dier saam geneem word op vakansie of nie).

(ii) Daar is ook gelet op die duur en die frekwensie van elke aktiwiteit.

(iii) Die betekenis van die spesifieke dier vir die eienaar en die keuse van 'n spesifieke ras.

Die vraelys is eenvoudig en bondig gehou vir praktiese doeleindes. Alle gegewens is op kwantitatiewe wyse deur middel van inhoudsanalise ontleed. In hierdie ondersoek is klem geplaas op enkele woorde, sinne en paragrawe wat herhaal of beklemtoon is. Hieruit is temas opgebou wat van belang is in 'n spesifieke konteks. Hierdeur is 'n geïntegreerde interaksiepatroon opgestel, waaruit betekenis verkry kon word uit die funksies wat geselskapsdiere vervul met die oog op groter begrip vir die menslike aspekte wat deel vorm van die veeartspraktyk.

RESULTATE

Die resultate word aangebied deur eerstens die veearts, kliënt en pasiënt in 'n beskrywing te identifiseer. Tweedens word die tema wat verkry is uit 'n kort beskrywing bespreek. Laastens word die rol van die veearts en geselskapsdier ten opsigte van die kliënt beskryf.

EERSTE KONSULTASIE

1. Beskrywende identifikasie

Die veearts; Die veearts is 'n dame in 'n voorstedelike praktyk en haar konsultasiekamer vorm deel van haar woonhuis.

Die kliënt: Kliënt A is 'n getroude dame in haar laat dertigs, en het geen kinders nie. Ten tye van die konsultasie met haar geselskapsdier was sy nie vergesel van haar eggenoot nie.

Die kliënt behoort tot die hoë sosio-ekonomiese groep en besit 'n groot aantal honde, katte en visse. Dit het geblyk dat sy 'n groot diereliefhebber is. Met die aanvanklike ontmoeting het dit geblyk dat sy baie betrokke is by haar dier wat sy aangebied het vir konsultasie.

Die pasiënt: Die pasiënt is 'n volwasse mannetjieskat. Die naam van die kat is Eros (die god van liefde). Die diagnose van die kat se siekte was gevorderde nierversaking en die veearts meld dat dit 'n terminale siekte is.

2. Inhoudsanalise

Gedurende die konsultasie het daar twee elemente in die kliënt se verhouding met haar diere na vore getree. Teenstrydighede ten opsigte van die funksie van die geselskapsdiere het by haar voorgekom en sy het nie geblyk bewus daarvan te wees nie. Hierdie paradoks word baie duidelik aangetoon in die temas wat bespreek word.

Tema I: Paradoks:- Betrokkenheid teenoor vrees vir nabyheid

Kliënt A is intens betrokke by haar diere maar terselfdertyd blyk daar by die kliënt 'n vrees vir nabyheid te wees.

Sprekend hiervan is die moeite wat sy doen met die voedselvoorbereiding vir haar diere en die feit dat sy op hierdie stadium weer die veearts nader omtrent 'n moontlike oorsaak van haar dier se siektetoestand. Daar kan uit die antwoord van die veearts afgelei word dat die diagnose van gevorderde nierversaking reeds in 'n vorige konsultasiesessie aan die kliënt meegedeel is. Dit blyk dat die naderende dood van haar dier vir die kliënt baie traumaties is. Sy kom gespanne en terneergedruk voor en sê ook reguit dat die situasie haar depressief maak. Dit kom voor asof die kliënt steeds die dier wil beskerm en sekuriteit by die dier soek, deur hom voortdurend vas te hou en met hom kontak te maak, gedurende die konsultasiesessie.

Toe die veearts meld dat die dier moontlik "uitgesit" (genadedood) moet word, het die kliënt die woord "uitsit" vermy en dit glad nie herhaal nie. Sy het doelbewus die gesprek in 'n ander rigting gestuur. Sy vestig die veearts se aandag op die simptome van die dier en skep moontlik so afstand tussen haarself en haar gevoelens oor die komende dood van die geselskapsdier. Die konsultasie blyk op 'n minder bedreigende vlak gehou te word, omdat die dier se dood moontlik vir die kliënt te emosioneel gelaai is. Die betekenis van bovermelde paradoks, dui moontlik op 'n ambivalente gevoel ten opsigte van die kliënt se siening van haarself. Aan die een kant hou sy 'n ideale beeld voor, as 'n mens wat betrokkenheid en meegevoel toon teenoor ander persone en diere asook 'n versorger en redder wat onafhanklik en selfgeldend kan optree. Andersyds is daar die ware beeld van die kliënt, waaroor sy moontlik ongemaklik voel. Sy skram telkens weg van nabyheid en kontak met persone wat te nou by haar betrokke wil wees. Sy blyk 'n mens te wees wat geliewers op bloot fisiese vlak aandag aan haar diere wil gee, omdat sy moontlik hierdie tipe verhouding as minder bedreigend beskou. Deur haar dominerende houding voel die kliënt moontlik meer in beheer van die situasie en kan sy moontlik haarself daardeur veilig laat voel ten opsigte van die nabyheid van ander mense.

Die funksie van die diere kan gesien word as 'n wyse waarop 'n gevoel van veiligheid by die kliënt geskep word, wat haar moontlik kan beskerm teen 'n kwesbaarheid ten opsigte van nabyheid. Die kliënt kom baie selfversekerd voor wanneer sy haarself bevind in die rol van 'n betrokke, versorgende moeder. Wanneer sy op 'n dieper vlak, as gevoelsmens, moet besluit oor die lot van haar geselskapsdier, skram sy hiervan weg. Dit blyk dat hierdie emosioneel-gelaaide besluit haar ongemaklik laat voel.

Tema II: Paradoks;— Aardsmoeder teenoor kinderloosheid

'n Ander tema wat herhaaldelik voorkom is dié van 'n kinderlose vrou wat terselfdertyd die rol van 'n aardsmoeder vervul. Die kliënt sê onomwonde dat die diere haar kinders is, omdat sy self nie in staat is om kinders te hê nie. Sy noem dat die diere haar behoefte aan kinders bevredig omdat dit haar die geleentheid gee om haar versorgingsbehoefte ("mothering") te bevredig. Sy weerspieël tergelykertyd die beeld van 'n "ideale moeder" maar toon ook tekortkominge in die versorging van haar diere. Dit kom voor asof die kliënt bevrediging daaruit put om haarself as 'n "redder van diere" te sien. Dit blyk dat die diere as kindersubstitute aangewend word en sodoende haar versorgingsbehoefte bevredig. Sy kan haar behoeftes verder bevredig deur die rol te vertolk van 'n moeder in 'n tehuis vir ouerlose kinders, in hierdie geval elenaarlose diere. Dit vorm ook deel van die aardsmoederbeeld wat aandag en erkenning van andere meebring. Sy sê pertinent dat sy elke dier gekies het op grond van unieke eienskappe maar toon paradoksaal weer op die vraelys aan, dat sy al haar diere as geskenke ontvang het. Dit blyk uit bykomende inligting verskaf deur die veearts, dat die mense van die omgewing verlore en rondloperdiere na haar neem vir versorging.

Oor die algemeen blyk die kliënt dominerend op te tree, maar neem tog verskeie kere 'n opvallend hulpelose, afhanklike houding in. Sy praat met vermeende kundigheid oor verskillende diëte van die diere maar is onkundig wanneer dit kom by die feit oor hoe lank die voedsel vars kan bly. Sy praat asof sy baie kennis besit oor sekere van die simptome, maar sy weet oënskynlik nie wat die rede vir die behandeling van die pasiënt is nie.

3. Die rol van die veearts

Gedurende hierdie konsultasie het die veearts 'n belangrike rol gespeel en het sy die nodige erkenning en goedkeuring aan die kliënt verskaf. Die gedrag van die veearts het moontlik die persoon se kwesbare ego versterk in die sin dat die kliënt se optrede van so 'n aard was dat die veearts net positief kon reageer en haar op so 'n wyse kon ondersteun en op haar gemak stel.

Die veearts was deurgaans bewus van die kliënt se behoeftes en het haar die nodige steun gegee.

Behoeftebevrediging is nagestreef deur die kliënt, deur gebruik te maak van die ondersteunende funksies wat van beide die geselskapsdier en die veearts verkry is. Dit blyk ook dat die kliënt op grond hiervan, baie egosentries ingestel is ten opsigte van haar diere en die veearts. As 'n voorbeeld hiervan kan genoem word dat sy inmeng met die veearts se verpligtinge teenoor ander kliënte. Sy voel dat haar probleem belangriker is as die veearts se praktykbestuur en ander kliënte se behoeftes.

4. Die rol van geselskapsdiere

Die diere dra by tot 'n moontlike verhoogde lewenskwaliteit vir die kliënt, veral ten opsigte van haar onbevredigde behoefte aan kinders. Die diere word as kindersubstitute aangewend. Sy kry deurgaans nog geselskapsdiere by wat moontlik kan dui op die bevestiging van haar versorgingsbehoefte asook die moontlikheid om op 'n meer omvattende wyse uitdrukking daaraan te kan gee. Die feit dat sy gedurig goedkeuring en erkenning vir die versorging van haar diere ontvang vanaf die veearts en ander diereliefhebbers, sluit moontlik hierby aan.

Die behoefte aan goedkeuring en erkenning is klaarblyklik vir haar belangrik. 'n Tweede behoefte wat moontlik bevredig word, is die positiewe onderskraging van haar selfbeeld. Hierdie funksie blyk uit die kliënt se reaksie op moontlike negatiewe kritiek. Sy noem onmiddellik 'n goeie daad wat sy aan diere verrig het aan die veearts om sodoende goedkeuring uit te lok. Dit blyk hieruit dat die kliënt nie van negatiewe kritiek hou nie en dit probeer vermy. 'n Derde funksie kan gesien word as die bevrediging van 'n sterk versorgingsbehoefte.

TWEEDE KONSULTASIE

1. Beskrywende identifikasie

Die veearts: Dieselfde veearts is betrokke

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as by die eerste konsultasie.

Die kliënt: Kliënt B is 'n ongetroude middeljarige dame, en kom uit die hoër sosio-ekonomiese groep. Sy is die eienaar van twee volwasse Maltesers en een klein tipe Franse Poedel. Al die honde is vroulik met eg vroulike name soos Chantal, Cherê en Candice.

Die pasiënt: Die kliënt bring 'n jong Franse Poedel na die veearts vir haar eerste inenting, maar bring ook die twee Maltesers saam vir diagnosering van moontlike diareë en wurmbesmetting.

2. Inhoudsanalise

Dit blyk dat die kliënt oorbetrokke is by haar honde en oormatige tyd en aandag aan hulle bestee. Sy is veral betrokke by die honde se kliniese tekens soos byvoorbeeld vae klagtes oor diareë met verdagte wurmbesmetting. Die honde tree vir haar op as tussengangers: Haar beheptheid met hulle fisiese welsyn skep moontlik die indruk dat sy baie vir die honde omgee en hulle baie goed versorg. Sy gebruik haar vorige werksondervinding (veterinêre laboratorium) om uit te brei oor die diere se fisiese aspekte en die besprekings hieroor blyk haar op haar gemak te laat voel. Dit is moontlik vir haar 'n veilige terrein om op te beweeg omdat dit bekend is en haar in beheer van die situasie laat voel. Alhoewel die betrokkenheid eg is, skyn dit oppervlakkig te wees omdat die honde soms deur haar optrede benadeel word. Die kliënt het die diere al oordoseer met wurmmiddels. Die veearts bevestig die feit dat sy die diere teen die eienaar moet beskerm, omdat die eienaar neig om hulle te oorbehandel.

Tema I: Paradoks:- Skynbare betrokkenheid teenoor oppervlakkigheid.

Dit blyk asof die kliënt nabyheid en kontak vermy, omdat sy dit moontlik moeilik mag vind om so 'n situasie te hanteer. Hierteenoor blyk dit dat die behoefte aan kontak steeds onbevredig is. Dit kan geïllustreer word deur die feit dat sy 'n derde hond aangeskaf het en ook dat sy die veearts weekliks kom besoek. Sy reageer skynbaar op 'n angstige manier wanneer sy met emosionele eise gekonfronteer word en toon op verbale en nie-verbale wyse aggressie. Dit kan gesien word in die feit dat sy die klein hondjie as parmantig beskou en hierdie houding verafsku. Daar is moontlik ook 'n mate van verwerping van hierdie hondjie by haar te bespeur wanneer sy hom as moeilik hanteerbaar beskryf in vergelyking met haar ander honde. Sy hanteer hierdie situasie deur 'n dominerende houding in te neem wat haar weer veilig en in beheer van die situasie laat voel. Die teenreaksie wat sy moontlik in andere uitlok, is skynbaar die van aggressie en verwerping. Die gevoelens van irritasie by die veearts wat teenoor die kliënt uitgespreek word, is 'n illustrasie hiervan. Haar toenemende besoeke aan die veearts dui moontlik daarop dat haar behoefte aan aandag en erkenning nie voldoende bevredig is nie. Dit blyk dat daar 'n vasgestelde interaksiepatroon by die kliënt teenwoordig is en sy moeilik hiervan kan afwyk. Hierdie interaksiestyl kan moontlik dui op 'n neurotiese aanpassing soos deur Horney[7] beskryf.

Tema II: Identifikasie met die vaderfiguur

Die kliënt meld dat sy haar honde as kinders beskou en lê, soos voorheen genoem, baie klem op hulle fisiese versorging. Hierdie aspek blyk ooreen te stem met die stereotipe beskouing van die vaderfiguur, waarin die bevrediging van affeksionele behoeftes nie op die voorgrond is nie. Haar kleredrag en houding mag aanduidend wees van 'n identifikasie met 'n manlike rol. Dit blyk dat die kliënt tans steeds 'n behoefte aan kontak met 'n vaderfiguur toon. Sy neem moontlik die rol van vader in teenoor haar honde, en blyk slegs oppervlakkig by hulle fisiese toestand betrokke te wees. Die kliënt kan moontlik ook met die honde identifiseer in die sin dat sy haarself kan sien in die rol van 'n klein dogtertjie teenoor 'n vaderfiguur. Die name van die honde blyk hierby aan te sluit. Dit wil voorkom asof die kliënt moontlik 'n probleem mag ondervind met die vroulike of moederrol. Die kliënt se moontlike behoefte aan nabyheid en aanvaarding spruit oënskynlik voort uit haar verhouding met haar ouers. Volgens die kliënt kan ouers 'n kind maak of breek.

3. Die rol van die veearts

Die kliënt benodig die veearts om vir haar leiding te gee oor haar diere en om erkenning en aandag te gee vir haar skynbare besorgdheid. Dit wil voorkom asof die kliënt haar eie behoeftes wil bevredig met die besoeke aan die veearts. Die werklike motivering vir die konsultasie blyk nie die siektetoestand van die diere te wees nie. Sy skep moontlik soms siektes om 'n konsultasie te regverdig en terselfdertyd bewondering af te dwing vir haar "onselfsugtige opofferings" teenoor die diere. Dit blyk in werklikheid asof hierdie houding teenoor die diere moontlik 'n egosentriese bevrediging van haar eie behoeftes kan wees. Die veearts beskryf haar rol in hierdie situasie as terapeutles van aard. Die kliënt word gerusgestel en ondersteun. Die veearts toon 'n aanvoeling vir die kliënt se situasie en identifiseer haar behoeftes. In die kommunikasie beperk sy die kliënt op 'n ferm, dog nie-veroordelende manier. Die kliënt beleef die veearts as 'n noodsaaklike steunsisteem en is afhanklik van die funksie wat die veearts en die diere vir haar vervul.

In hierdie geval het die veearts egter nie die interaksieproses soos deur die kliënt voorgeskryf, voortgesit nie. Spesifieke grense is gestel. Dit het tot gevolg gehad dat die kliënt nie die veearts se positiewe gesindheid kon misbruik nie. 'n Verdere gevolg blyk te wees dat die kliënt tot 'n mate vir die tydsduur van die konsultasie, verantwoordelikheid vir haar gedrag moes neem. Daar is egter nie 'n onrealistiese verwagting van die kliënt gekoester nie.

4. Die rol van geselskapsdiere

In die interaksie met haar diere verplaas die kliënt haar gevoelens op haar diere en blyk dit dat sy onbewus hiervan is. Sy toon min insig in haar gedrag waarvan die oordosering van haar diere 'n voorbeeld is. Sy neem nie verantwoordelikheid vir haar gedrag nie. Sy noem byvoorbeeld dat 'n eienaar sy dier kan "maak of breek". Sy toon ook aan dat die opvoeding deur ouers 'n kind se toekoms en gedrag bepaal, en dat die kind self nie 'n verantwoordelikheid daaromtrent het nie. Sy gebruik die diere as statussimbole en dring daarop aan dat hulle geregistreer moet wees. Die kliënt blyk bevrediging daaruit te put om goedkeuring van ander persone te verky. Dit blyk dat sy deurgaans die veearts, diere en 'n dieregedragskenner benodig om tydelike goedkeuring en oppervlakkige kontak te verskaf. Die diere se name en hul rasegtheid verteenwoordig vir die kliënt status, en kan moontlik vir haar bepalend wees van andere se siening van haarself. Die feit dat sy 'n opregte hond van 'n ander ras aangeskaf het, mag aanduidend daarvan wees dat sy meer aandag op haarself wil vestig.

DERDE KONSULTASIE

1. Beskrywende identifikasie

Die veearts: Die veearts is 'n dame in 'n voorstedelike praktyk. Die praktyk is weg van haar huis geleë.

Die kliënt: Kliënt C is 'n adolessente dogter vergesel van haar moeder. Die kliënt behoort tot die hoër sosio-ekonomiese groep. Die gesin besit twee Duitse Herdershonde en 'n Malteser. Die Malteser word spesifiek as die geselskapsdier van die dogter gesien. Sy het die hond as 'n geskenk van haar vriend ontvang.

Die pasiënt: Die pasiënt is 'n vroulike, volwasse Malteser. Die hondjie word vir haar jaarlikse inenting na die veearts gebring.

2. Inhoudsanalise

Die diere vertolk in die gesinsopset bloot die rol van geselskapsdiere. Hulle word in die algemeen nie aangewend om neurotiese behoeftes uit te leef nie. Die groter honde word aangewend as waghonde en die klein hondjie is die dogter se metgesel.

Tema I: Positiewe interaksie

Die dogter reageer spontaan en handhaaf haarself op interpersoonlike vlak met selfvertroue teenoor die veearts. Sy beantwoord die vrae en toon inisiatief deur self vrae te vra en voorstelle te maak. Sy het genoeg vertroue in haarself en erken indien sy 'n fout gemaak het. Emosionaliteit is nie vir haar of haar moeder bedreigend nie en hulle skroom ook nie om dit te toon nie. Hulle sê albei hoe lief hulle vir die hond is en hoe diep sy in hulle harte ingekruip het.

Die dogter het 'n gesonde siening van haarself wat haar in staat stel om in 'n vreemde situasie vol selfvertroue op te tree. Sy aanvaar kritiek sonder enige selfbewustheid of ooglopende ongemak. Vanuit die aard van die gesinslede se houding teenoor die hond as 'n geskenk van haar vriend, is dit duidelik dat haar verhouding goedgekeur word en haar keuse aanvaar word. Dit kan ook daarop dui dat sy as kind haar ouers se aanvaarding ten volle verkry het.

Die dogter word deur die ouers toegelaat om self keuses te maak. Sy kan byvoorbeeld self besluit of die dier gesteriliseer moet word of nie. Dit spreek moontlik haar verantwoordelikheidsin aan. In hierdie gesinskonteks blyk dit dat die dier haar help in die proses van volwassewording. Dit blyk verder dat die vryheid wat aan haar gegee word ten opsigte van keuses wat gemaak moet word oor die dier en die keuse van haar vriende, tot gevolg het dat sy met selfvertroue verantwoordelikheid kan neem. Uit die gesprek met haar moeder blyk dit ook dat sy die nodige ondersteuning en erken-

ning kry. Haar selfstandigheid en spontaneïteit in 'n vreemde situasie kan hieraan toegeskryf word.

3. Die rol van die veearts
In hierdie geval speel die veearts geen sielkundig ondersteunende rol nie en is bloot daar vir die verskaffing van inligting en diens. Die veearts toon aan dat die dier moontlike probleme mag ondervind gedurende dragtigheid en stel as oplossing sterilisasie voor. In die sosiale konteks gesien is beide die moeder en die dogter goed aangepaste individue met goeie interpersoonlike verhoudings binne en buite die gesin. Dit blyk uit hulle verhouding met die veearts maar ook uit hulle verhouding met mekaar. Hulle maak nie van diere of 'n besoek aan die veearts gebruik om hulle van hulp te wees ten opsigte van onvervulde emosionele behoeftes nie. Die veearts lewer dus primêr 'n veterinêr-kliniese diens.

4. Die rol van geselskapdiere
4.1 Die rol van die dier by die dogter
Die verhouding tussen die hond en die dogter is nie oorafhanklik of oorbetrokke van aard nie, maar berus op gesonde belangstelling. Die kliënt bring die hond omdat sy die dier se gesondheid in ag neem en vir haar omgee. Die dogter neem self die versorging van haar hond waar en sien dit op realistiese vlak. Sy besef haar verantwoordelikheid as eienaar en tree daarvolgens op. Die funksie van die hond as metgesel vir die dogter kan gedefinieer word as kameraadskap. Volgens die kliënt kan sy as adolessent persoonlike aangeleenthede en geheime met die hond deel wat sy op daardie stadium verkies het om nie met ander persone te deel nie.

4.2 Die rol van die dier in die gesinsopset
Die funksie van die dier in die gesin is 'n gemeenskaplike belangstelling en word deur beide die moeder en die dogter se houdings bevestig. Dit blyk dat die gesin se betrokkenheid by die dier hulle nader aan mekaar bring en hulle toon ook begrip vir mekaar. Uit die gesprek tussen moeder en dogter blyk dit dat 'n spontaneïteit tussen die gesinslede bestaan. Dit kan aandui dat hulle 'n oopheid in hulle verhoudinge met mekaar handhaaf.

Bogenoemde kan afgelei word uit die feit dat die gesinslede almal besorg was dat die kleinrastefie, deur een van die groot Duitse Herdershonde tuis, gedek kon gewees het. In hierdie gesin blyk die rol van die dier, bloot geselskapsdier te wees sonder enige onderliggende "neurotiese" konnotasies daaraan verbonde.

VIERDE KONSULTASIE
1. Beskrywende identifikasie
Die veearts: Dieselfde veearts is betrokke as by die derde konsultasie.

Die kliënt: Kliënte D is 'n middeljarige egpaar en behoort tot die midde sosio-ekonomiese groep. Hulle kinders het reeds die huis verlaat. Hulle beskou hulleself as hondetelers en besit drie Rottweilers en een Malteser. Twee van die Rottweilers is manlik en hulle name is Hitler en Rommel.

Die pasiënt: Die pasiënt is 'n Rottweilerteef wat vir teeldoeleindes gebruik word. Die rede vir die besoek was 'n bloederige vaginale afskeiding by die hond. Monsters is versamel en weggestuur vir laboratoriumondersoeke om die oorsaak van die afskeiding vas te stel. Die voorlopige diagnose was 'n aborsie.

2. Inhoudsanalise
Daar is 'n spesifieke verhouding tussen die man en die Rottweilers, wat in die bespreking toegelig sal word. Die vrou het weer op haar beurt 'n spesifieke verhouding met die Malteser.

Tema I: Gesinspanninge
Dit wil voorkom asof die vrou meer van die honde gedistansieer is as die man en slegs oppervlakkig betrokke is by die groot honde. Sy toon 'n teenstrydigheid in haar houding teenoor hulle en is onderliggend aggressief. Sy neem hulle fisiese versorging waar, maar is deurgaans geïrriteerd met die take wat sy moet uitvoer, soos die skoonmaak van die diere se hokke. Dit blyk dat die vrou jaloers is op die verhouding wat haar man met sy honde het. As reaksie hierop het sy vir haar 'n malteser aangeskaf. Dit mag wees omdat die honde soveel aandag van haar eggenoot kry en omdat sy self 'n behoefte aan sy aandag en erkenning het.

Die kliënt het verskeie klagtes oor haar eie gesondheid gelug soos byvoorbeeld dat sy soms voel asof haar keel kan toetrek, veral as sy die vuil hokke moet skoonmaak. Hierdie klagtes was moontlik 'n geleentheid wat sy vir haar man geskep het om by haar betrokke te wees en aandag aan haar te gee. Die dominerende houding waardeur sy aandag wil verkry, veroorsaak egter dat haar man steeds verder terugtrek en minder aan hierdie else voldoen. Dit word geïllustreer deur hulle woordewisseling tydens die konsultasie waar die vrou die man die stryd aangesê het. Die man het skaars geantwoord. Hierdie optrede van die vrou teenoor haar man laat hom blykbaar op 'n sekere wyse reageer, naamlik om meer passief te raak. Dit blyk dat die vrou moontlik probeer om deur haar dominerende houding 'n dieperliggende afhanklikheid weg te steek. Wanneer sy nie aandag ontvang nie en die teenoorgestelde gedrag by hom uitlok, voel sy gefrustreerd en magteloos. Hierdie gevoel word uitgebeeld deur haar sarkastiese en snedige opmerkings teenoor haar man.

Tema II: Die driehoekeffek. ('n derde party wat spanning ontlaai)
'n Gevolg van hierdie interaksie lê daarin dat die egpaar deur hulle aangeleerde kommunikasiepatrone ten opsigte van die diere, elkeen sy eie behoefte uitleef teenoor die diere en minder by mekaar betrokke is. Dit blyk uit die feit dat die man sy spesiale honde het en die vrou haar spesifieke hond het. Dit mag ook wees dat die betrokkenheid by die diere, 'n gemeenskaplike aanknopingspunt is en die element mag wees wat die egpaar bymekaar help hou. Kommunikasie vind dikwels deur 'n derde party plaas en direkte interpersoonlike spanninge word na die derde party herlei. Hierdeur word direkte konfrontasie deur die twee partye vermy.

3. Die rol van die veearts
Dit blyk dat die egpaar 'n behoefte toon aan 'n persoon buite die gesinsopset, wat vir hulle rigtyne kan neerlê en verantwoordelike besluite kan neem. Die veearts toon dan ook begrip vir die egpaar se probleme en gee aan hulle die nodige ondersteuning. Wanneer die veearts wel sekere voorstelle maak, reageer die egpaar verlig hierop en dit verhoed 'n moontlike probleemsituasie tussen die twee partye. Dit blyk hieruit dat hulle wel hulle samewerking sal gee aan 'n neutrale persoon wat begrip toon vir hulle probleme. Die veearts het suksesvol as bemiddelaar tussen die twee partye opgetree en so 'n rigtinggewende rol vervul. Die veearts se optrede in hierdie situasie was gewens en effektief.

4. Die rol van die geselskapsdiere
Dit blyk dat die verhouding tussen die man en sy hond heg is en dat hy baie besorgd is oor haar. Hy is ook trots op die honde en sien hulle as 'n voortsetting van sy manlike rol (andromorfisme). Die name van die manlike honde illustreer hierdie stelling. Wat interaksie betref, was hy meer op die agtergrond tydens die konsultasie en 'n element van eg emosionele betrokkenheid het voorgekom. Hy toon nie overte aggressiewe gedrag teenoor sy vrou, wanneer sy hom op dominerende, vyandige wyse aanspreek nie. Dit mag daarop dui dat hy sy aggressiewe gevoelens onderdruk en in sy kommunikasie met haar beheersd en teruggetrokke voorkom. Die honde weerspieël elenskappe wat hy onderdruk en mag presies uitbeeld hoe hy graag sou wou optree. Hy projekteer sy aggressie teenoor sy vrou op sy honde. Dit kan gesien word in die feit dat een van sy honde op 'n keer sy vrou gebyt het en hy dit as amusant beskou het. Op die vraelys het die vrou aangedui dat die honde as hulle kinders gesien word. 'n Moontlike funksie wat die honde kan vervul, naamlik om 'n mate van kommunikasie tussen die egpaar te bewerkstellig, kon vroeër deur die kinders vervul gewees het. Hierdie moontlike funksie kon in werklikheid afstand tussen die twee egliede geskep het. Alternatiewelik kon die honde moontlik tot 'n mate teenoor mekaar afgespeel word om die egpaar se ele behoeftes te bevredig.

BESPREKINGS
By al 4 konsultasies word daar gekyk na die interaksiepatrone tussen die veearts, kliënt en pasiënt. Die verhoudings het gewissel in aard en intensiteit van betrokkenheid van die kliënte teenoor hulle diere. Kliënte A, B en die eggenote in D was oënskynlik bloot oppervlakkig by hulle geselskapsdiere betrokke. Kliënt C en die eggenoot in D was realisties en opreg by hulle diere betrokke.

Besliste temas in die interaksiepatrone tussen eienaars en geselskapsdiere kon geïdentifiseer word. Die temas is die volgende:

(i) Paradoks:- Betrokkenheid teenoor vrees vir emosionaliteit.
(ii) Paradoks:- Aardsmoeder teenoor kinderloosheid.
(iii) Paradoks:- Skynbare betrokkenheid teenoor oppervlakkigheid.
(iv) Identifikasie met vaderfiguur.
(v) Positiewe interaksie.
(vi) Gesinspanninge.
(vii) Driehoekeffek.

Hierdie temas gee inhoud aan die interaksiepatrone tussen geselskapsdiere en veeartskliënte. Brickel[3] toon aan dat diere in terapeutiese situsies benut kan word om interaksie tussen persone te insleer. Verder kan die geselskapsdier ook aangewend word om persone

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behulpsaam te wees in die uitdrukking van onaanvaarbare gevoelens. Dit word beklemtoon dat geselskapsdiere die balans in die geestesgesindheid van individue kan bevorder. 'n Geselskapsdier kan dus as katalisator optree in interpersoonlike verhoudings[10]. Die inhoud van die interaksiepatrone tussen pasiënt en kliënt wat beskryf is, toon aan dat die menslike aspek in die veeartspraktyk ook belangrik is[1].

Die funksies van die veearts in die konsultasiekamer kan nou as volg beskryf word:

(i) 'n Veterinêr-kliniese diens wat die pasiënt direk bevoordeel.

(ii) 'n Algemene ondersteunende rol ten opsigte van die kliënt se emosionele behoeftes[4]. Dit is 'n sielkundig-ondersteunende diens.

(iii) 'n Verwysingsrol ten opsigte van ernstiger emosionele behoeftes van kliënte. Dit kan op twee maniere geskied. Eerstens kan die veearts 'n sielkundige konsulteer oor die wyse waarop sulke probleemkliënte beter hanteer kan word en tweedens kan die veearts in uitsonderlike probleemgevalle in samewerking met die kliënt, die kliënt direk na 'n sielkundige verwys.

In hierdie ondersoek blyk dit dat kliënte in 3 van die konsultasies naamlik kliënte A, B en die egpaar in D, moontlik baat kon vind by ondersteunende terapeutiese leiding.

Om 'n ondersteunende diens te kan lewer, is multidissiplinêre samewerking tussen natuurwetenskappe en die geesteswetenskappe 'n vereiste. Om hierdie samewerking te bewerkstellig moet die veearts opgelei wees om emosionele behoeftes van kliënte te kan identifiseer. Die klem in hierdie opleiding van veeartse ten opsigte van menslike gedrag, moet eerder val op 'n bewustheid en sensitiwiteit vir menslike behoeftes eerder as op diepgaande analisering van persoonlikhede. Die veearts moet dus nie die rol van 'n kliniese sielkundige probeer vervul nie. In die lig van die rol wat veeartse ten opsigte van hulle kliënte (dit is die menslike aspek van 'n praktyk) as professionele persone moet speel, is dit dalk nodig om die keuring van veterinêre studente te heroorweeg. Die keuring moet nie nét op grond van akademiese prestasies plaasvind nie, maar behoort ook die potensiaal van hierdie studente om met kliënte te kommunikeer in ag te neem. Dit beteken dat die student uiteindelik geskik moet wees om sinvol met sy kliënt te kan kommunikeer en 'n algemene ondersteunde rol ten opsigte van die kliënt se emosionele behoeftes te kan vervul. Die veearts moet ook ernstige gevalle van emosionele probleme kan identifiseer vir verwysing. Op dié wyse kan die veearts sy gemeenskapsdiens uitbrei. Daar moet egter met hierdie ondersoek in gedagte gehou word dat dit slegs 'n beskrywende studie behels waaruit moontlike gevolgtrekkings gemaak kan word. Verdere navorsing wat 'n verteenwoordigende steekproef insluit word aanbeveel. Die waarde van hierdie studie lê daarin opgesluit dat die interaksie tussen die eienaar, geselskapsdier en veearts in die praktyksituasie sekere konsepte uit die literatuur bevestig het.

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A STATISTICAL METHOD TO EVALUATE THE RELIABILITY AND REPRODUCIBILITY OF A STANDARDISED TECHNIQUE FOR THE CONDUCT OF ANTIBIOGRAMS ON TWO SPECIES OF ENTEROBACTERIACEAE

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ABSTRACT

Antibiograms are only as reliable as is permitted by the quality of the specimen, the reliability or reproducibility of the method used and the ability of the clinician to interpret the results. Non-standardised methods may give erroneous results. Some of these procedural effects were investigated by means of statistical analysis of variance to determine the effect on the reliability of the test. To ensure reliable and reproducible test results with antibiograms, it is imperative that the type of sensitivity test medium as well as the density of the inoculum of the test organisms be standardised.

Key words: Antibiogram, reliability, reproducibility, analysis of variance, standard error of measurement

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INTRODUCTION

Every antimicrobial drug is normally active against a defined range of diverse micro-organisms. Certain organisms are consistently sensitive while others are resistant to the same drug. For instance, anaerobic bacteria are invariably sensitive to metronidazole or its analogues, whereas these drugs have no effect on aerobic organisms[8]. On the other hand, different isolates of a specific organism like *Streptococcus faecalis* or *Staphylococcus aureus* vary in their sensitivity to a given antimicrobial drug[6]. When resistance develops during the course of treatment, the desired therapeutic effect is not achieved in the patient.

Bacterial resistance may therefore be regarded as one of the main obstacles to the therapeutic use of many antibiotics. For this reason, determinations of bacterial resistance or sensitivity are essential prerequisites for the rational use of antibiotics and other chemotherapeutic agents and for the preservation of the efficacy of this important group of therapeutic substances. They are also indispensable in the evaluation and comparision of new antimicrobial drugs and in certain epidemiological studies. The results of such determinations are, however, not absolute values because they are influenced by many variables in the techniques and conduct of the specific tests[4].

The reason for erroneous results may be found in the use of non-standardised methods, the haphazard execution of such methods or an incorrect interpretation of the results[5]. These may furthermore be the reason for a lack of correlation between the result of an in vitro test and the in vivo therapeutic effect[10]. Table 1 summarises some of the differences in antibiogram testing procedures and techniques in various laboratories. Variations in the type of medium, the density and method of spreading the inoculum, and the length of the incubation period are of specific importance.

In 1966, Bauer et al.[1] attempted to standardise a single-disc sensitivity test method and established interpretative data on the diameter of inhibition zone and related this to the criteria, resistant, intermediate or sensitive. For most antimicrobials there is a linear relationship between zone size and the log minimum inhibitory concentration. This observation unfortunately led to the common belief that the medium giving the largest average zone of inhibition with an antimicrobial drug after a number of repetitions of sensitivity tests on reference organisms, will necessarily be the best.

The assessment of antimicrobial sensitivity by means of the agar diffusion technique is currently commonly used as a routine method[4] because in contrast to the agar dilution technique, it is a very simple and rapid procedure. However, it will only give reliable and reproducible quantitative results when conducted in an exact and standardised manner. Except for the inherent differences in the susceptibility of bacteria, all the other factors which can influence the size of the inhibition zones, should therefore be kept meticulously constant. Because of the vast differences between micro-organisms, the response of many to the same experimental treatment (antibiogram) may show large variability. This variability is largely attributable to the differences between various organisms before execution of the antibiogram. If this source of variability is separated from procedural effects and experimental error, the sensitivity of the technique is increased. If it cannot be estimated, it remains part of the uncontrolled sources of variability and is thus automatically part of the experimental error[11].

Differences in procedural effects influence the reliability of results obtained in different laboratories[5]. These discrepancies cause a fluctuation in the size of the zone of inhibition of growth of the same organism, obtained with different in vitro agar diffusion tests even when employing discs with a specified content of the same antibiotic. It was therefore decided to use statistical methods to investigate the reliability of a standardised technique for the conduct of antibiograms on Gram negative enterobacteria and to determine the effect of different variables on the reliability of the test.

MATERIALS AND METHODS

Conduct of the sensitivity test

The principles of an agar diffusion technique, which had been exhaustively studied over a period of 10 years by an International Collaborative Study Group of the WHO[4], was used with minor modifications. This is comparable with the technique employed by Buys et al.[2].

Bacterial cultures

The following bacterial cultures were checked for purity and identity, freeze-dried and reconstituted as required:

E. coli NCTC 10418 (a standard sensitive strain internationally used as a control in antibiograms)

E. coli J162N, and

Salmonella LT_2 trp 8/N

Media

Dehydrated Oxoid Mueller-Hinton agar (MHA) and Oxoid Iso-Sensitest agar (ISA) were reconstituted according to the manufacturer's instructions. A volume of 30 ml of medium was dispensed into 90 mm disposable petri dishes, to ensure a constant depth of approximately 4 mm of the agar medium.

Inoculum

The freeze-dried cultures were activated by inoculation into nutrient broth (Oxoid) and incubated overnight at 37°C. A drop of culture was transferred with a sterile syringe to 9 ml of nutrient broth. In the standard technique this was incubated at 37°C for approximately 18 h and then diluted 5×10^{-3} in nutrient broth at room temperature. This dilution was used as the inoculum and it consistently caused a dense but not confluent growth on the agar surface[3]. To determine the influence that variations in the density have on the reliability of the results, suspensions of *E. coli* J 62/N were

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also diluted 1 x 10^{-4} and used as inoculum.

Spreading the inoculum
The sensitivity plates were allowed to equilibrate with room temperature before inoculation to prevent uneven absorption of the inoculum into the agar. A volume of 0,7 ml of the culture dilution[2] was applied to every plate and spread evenly with a sterile bent glass rod. The excess was drained to the side of the plate and removed aseptically with a syringe. The plates were then kept at room temperature for approximately 10 m to dry thoroughly before the antimicrobial discs were applied.

Antimicrobial drugs and discs
The concentrations of the different antimicrobial agents in the "Mast"- sensitivity discs are shown in Table 2. These discs have a diameter of 6,5 mm. The containers with the different discs had been stored in a deep freeze and on removal allowed to equilibrate with room temperature before being opened, to avoid condensation on the discs[5]. The individual discs were firmly applied to the agar surface in a fixed pattern with 5 discs around the circumference and 1 disc in the centre of the plate. This allowed for a distance of 25 mm between any 2 adjoining discs, and 12,5 mm between any disc and the edge of the plate in order to prevent overlapping of zones of inhibition.

The inoculated plates were incubated at 37°C for 24 h before the diameter of the zone of inhibition around each disc was accurately measured to the nearest millimeter with the aid of a "Mast ring -S" zone reader.

Statistical analysis of data
Coefficient of variation
The magnitude of the variation of zone sizes obtained with specific antimicrobial drugs, due to different media, was determined for each antimicrobial drug on both MHA and ISA by calculating the coefficient of variation (C.V.)[9]. The C.V. is simply the standard deviation expressed as a percentage of the mean according to the formula $C.V. = (S_{n-1})100 / \bar{x}$

Analysis of variance
A single-factor experimental design with repeated measurements on the same elements was employed[11]. Table 3 illustrates the notation for this type of design in terms of the various repetitions of the antimicrobial sensitivity test with a particular antimicrobial drug at different times, as the elements of the statistical sample. The first subscript to an x shows the number of the antimicrobial drug for which the measurements were recorded, and the second subscript the repetition number of the test. Thus, the symbol x_{1i} represents the size of the zone of inhibition in relation to antimicrobial drug 1 on repetition i of the test. The symbol P_i represents the sum of k entries in row i. The mean of the measurements on drug i after k repetitions of the test is $\bar{P}_i = P_i/k$. The symbol T_k represents the sum of n measurements for all the antimicrobial drugs during repetition k and is equivalent to totalling all entries in a single column. The mean of the n measurement for repetition k of the test, is designated as $\bar{T}_k = T_k/n$. The sum of the kn measurements in the experiment is designated as

Table 1. **Differences in antibiogram testing procedures and techniques between different laboratories**

A.	**GROWTH MEDIUM**	
I	Defined sensitivity test medium	
	(a) Enrichment:– Whole blood	Equine
	Chocolated blood	Ovine
	Haemolised blood	Bovine
	(b) No enrichment	
	(c) Depth of agar:– Constant	
	Variable	
II	Undefined medium	
B.	**INOCULUM**	
I	Type :– Primary culture	
	Pure culture	
II	Density :– Constant	
	Variable	
III	Spreading on plate :– Swab	
	Bacterial loop	
	Flooding	
IV	Type of growth :– Confluent	
	Dense but not confluent	
	Well separated colonies	
C.	**DISCS**	
I	Type :– *Multodisc*	
	Single	
II	Spectrum of antimicrobial agents	
III	Drug Content :– Standardised — high concentration	
	— low concentration	
	Variable	
D.	**KNOWN SENSITIVE CONTROL ORGANISMS**	
I	Used	
II	Not used	
E.	**INTERPRETATION**	
I	Reference standards	
II	Arbitrary	

Table 2. **The concentrations of the different antimicrobial agents in the "Mast"-sensitivity discs**

Ampicillin	10 µg	AP_{10}
Streptomycin	10 µg	S_{10}
Kanamycin	30 µg	K_{30}
Neomycin	10 µg	NE_{30}
Gentamycin	10 µg	GM_{10}
Tetracycline	25 µg	T_{25}
Chloromycetin	25 µg	C_{25}
Sulphafurazole	200 µg	SF_{200}
Trimethoprim	2,5 µg	$TM_{2,5}$
Furazolidone	200 µg	FZ_{200}

$G = \sum_{x=1}^{k} T_x = \sum_{i=1}^{n} P_i$ and the grand mean of all the measurements is designated as

$$\bar{G} = G/kn = \sum_{i=1}^{n} \bar{P}_i/n = \sum_{x=1}^{k} \bar{T}_x/k.$$

For the analysis of variance the total variation is divided into two parts: One part is a function of differences between the means of the different antimicrobial drugs; the other part is a function of the pooled variation within one antimicrobial drug.

The total variation was calculated as $SS_{total} = \Sigma\Sigma(X_{ix} - \bar{G})^2$. This source of variation has $kn - 1$ degrees of freedom. The part of the total variation due to differences between the means of the drugs was calculated as $SS_{b.drugs} = k\Sigma(\bar{P}_i - \bar{G})^2$. This source of variation may be viewed as to be due to the differences between all possible pairs of $\bar{P}_i$. The larger such differences are, the larger will be this source of variation. Since there are n means, this source of variation has $n - 1$ degrees of freedom.

The variation within a drug i was calculated as $SS_{w.drug\,i} = \Sigma(X_{ix} - \bar{P}_i)^2$, the sum of the squared deviations of the observations on drug i about the mean for that drug. This source of variation has $K - 1$ degrees of freedom. The pooled within-drug variation with $n(k - 1)$ *degrees of freedom, was calculated as* $SS_{w.drug} = \Sigma SS_{w.drug\,i} = \Sigma\Sigma(X_{ix} - \bar{P}_i)^2$. It is readily shown that the between- and within-drug sources of variation are statistically independent[11] and that $SS_{total} = SS_{b.\,drugs} + SS_{w.drug}$. The degrees of freedom corresponding to these sources of variation are also additive, hence $kn - 1 = (n-1) + n(k - 1)$.

The pooled within-drug variation is divided into two parts: one which depends upon differences between the repetition means, and a second which consists of residual variation. That part which depends upon differences between repetition effects, with $k - 1$ degrees of freedom, was calculated as $SS_{repetition} = n\Sigma(\bar{T}_x - \bar{G})^2$. The residual variation with $(k - 1)(n - 1)$ degrees of freedom was calculated as $SS_{residual} = \Sigma\Sigma[(X_{ix} - \bar{G}) - (\bar{P}_i - \bar{G}) - (\bar{T}_x - \bar{G})]^2$. The terms that were subtracted from $X_{ix} - \bar{G}$ are, respectively, the drug and repetition effects so that the residual variation represents those sources of variation in the total that cannot be accounted for by differences between the drugs and differences between the repetitions. The analysis of the sources of variation and the corresponding degrees of freedom are shown schematically in Figure 1.

Part (i) of Table 4 summarises the formulas used for the definitions of the sources of variation. The formulas (1), (2), and (3) are identical to those used in the case of single-factor experiments which do not have repeated measures. Formula (4) occurs only in experiments having repeated measures. In each case the divisor in a term is the sum of the number of observations, to obtain an element in the numerator. For example, G is the sum of kn observations, T_x is the sum of n observations (in repetition x) and P_i is the sum of k observations (for antimicrobial i). Part (ii) of table 5 outlines a summary of the analysis of variance appropriate for this design. Mean squares (MS) were obtained from corresponding sums of squares by dividing the latter by their respective degrees of freedom.

The results of all the repetitions of the diffusion tests conducted with the test organisms were subjected to an analysis of variance[11] according to the above methods and from this the reliability[7] was calculated to enable a comparison of the different results. The reliability of the average size of the inhibition zones of all the drugs tested with each repetition of a sensitivity test on a specific organism, was calculated as the reliability coefficient r_n. Similarly r_1 was calculated as the reliability coefficient of the average size of the zones of inhibition of all the drugs in a single sensitivity test. The formulas used for this were

$r_n = n\theta/(1 + n\theta)$ and $r_1 = \theta/(1 + \theta)$

where the term $\theta = (MS_{b.drugs} - MS_{w.drugs})/kMS_{w.drugs}$.

The standard deviation $(S_n - 1)$ of the size

Table 3. **Notation for a single-factor experimental design with repeated measurements on the same elements**

Anti-microbial drug used.	Number of the repetition of the test (x)				Total	Mean
(i)	1	2	...	k		
1	X_{11}	X_{12}	.	X_{1k}	P_1	$\bar{P}_1$
2	X_{21}	X_{22}	.	X_{2k}	P_2	$\bar{P}_2$
.	.	.	.	.	.	.
n	X_{n1}	X_{n2}	.	X_{nk}	P_n	$\bar{P}_n$
Total	T_1	T_2	.	T_k	G	
Mean	$\bar{T}_1$	$\bar{T}_2$	.	$\bar{T}_k$		$\bar{G}$

$P_1 = \sum_{x=1}^{k} P_{ix}$, for $i = 1$ $\quad$ $\bar{P}_1 = \sum_{x=1}^{k} P_{1x}/k$ $\quad$ $T_1 = \sum_{i=1}^{n} T_{ix}$, for $x = 1$ $\quad$ $\bar{T}_1 = \sum_{i=1}^{n} T_{i1}/n$

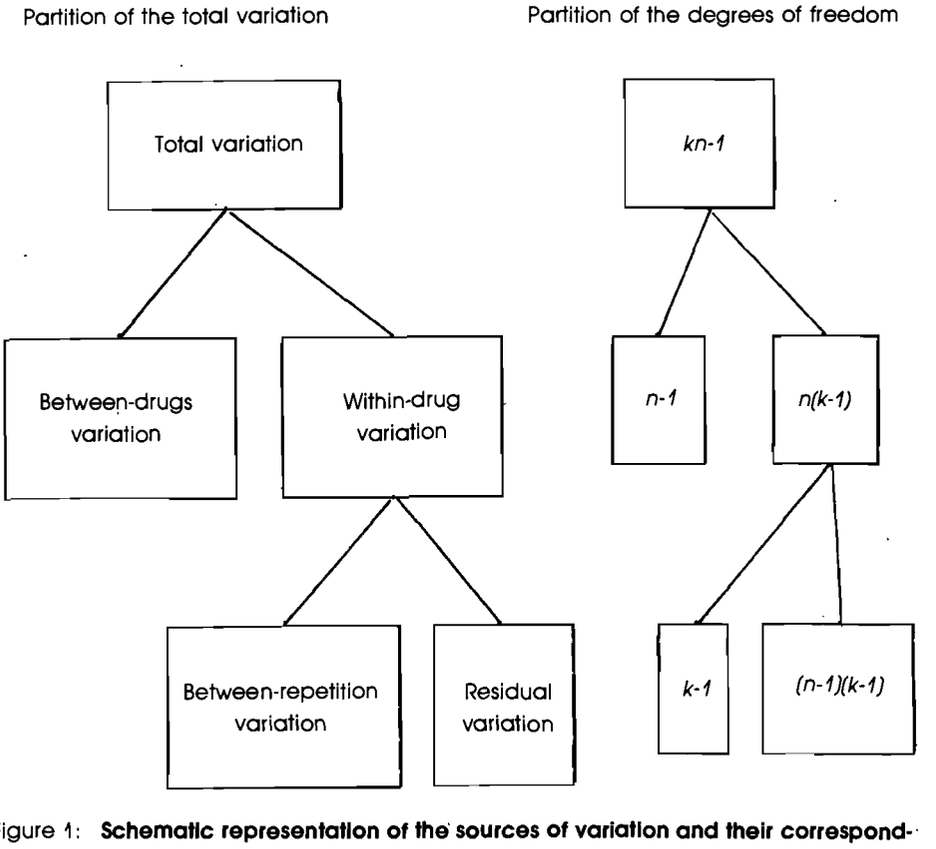

Figure 1: **Schematic representation of the sources of variation and their corresponding degrees of freedom used in the analysis**

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Table 4. **Summary of computational procedures**

(i)	(1) = G^2/kn (2) = $\Sigma\Sigma X^2$ (3) = $[\Sigma(T_x)^2]/n$ (4) = $[\Sigma(P_1)^2]/k$			
	Source of variation	SS (variation)	df (degrees of freedom)	MS (mean squares)
(ii)	Between drugs	$SS_{b.drugs} = (4)-(1)$	n-1	$SS_{b.drugs}/n-1$
	Within drug	$SS_{w.drug} = (2)-(4)$	n(k-1)	$SS_{w.drug}/n(k-1)$
	Repetitions	$SS_{repetitions} = (3)-(1)$	k-1	$SS_{repetitions}/k-1$
	Residual	$SS_{residual} = (2)-(3)-(4)+(1)$	(n-1)(k-1)	$SS_{residual}/(n-1)(k-1)$
	Total	$SS_{total} = (2)-(1)$	kn-1	

of inhibition zones, in every repetition with a specific test organism was determined and together with the calculated value of r_1 used to calculate the standard error of measurement (s_e) of a single test[7] according to the formula $s_e = (s_{n-1})\sqrt{(1-r_1)}$. The reliability coefficient r_1 rather than r_n were used in the calculations of the s_e of a single test within a series of repetitions, to allow a better comparison between results. This is more comparable to the practical situation where the reliability of a single sensitivity test is relevant[7].

RESULTS

The results of the effect of different media on the C.V. and average size of the zones of inhibition with the test organism are given in Table 5. The effect of a variable medium on the standard measurement of error of the size of the inhibition zones is presented in Table 6. The effect of variable inoculum densities of different test organisms using a constant medium, on the standard measurement of error of the size of the zone of inhibition is depicted in Table 7.

DISCUSSION

When the C.V. of the sizes of the zones of inhibition is calculated it can be used to compare the percentage of variation of zone sizes of different tests independent of the magnitude of the average zone size. From the results of comparative tests conducted with *E.coli* NCTC 10418 (Table 5) it is evident that with the exception of ampicillin, the variation of inhibition zone sizes of the other drugs tested was bigger on MHA than on ISA. Therefore, ISA will give more reproducible results with the drugs in question, despite the fact that the average size of zones of inhibition were not necessarily bigger on ISA. In fact the average inhibition zones with streptomycin, gentamycin and tetracycline were smaller on ISA than on MHA but the C.V.'s on ISA were less than on MHA. The findings of Buys et al. [2] could also be confirmed that sulphafurazole, furazolidone and neomycin gave larger and tetracycline smaller zone sizes on ISA.

In many laboratories standardised sensitivity test media are not used[2], but rather blood agar which varies considerably in different batches with regard to the variables already stated. To simulate such circumstances, the zone sizes for each drug on both MHA and ISA were combined. The calculated C.V. for these combined data (Table 5) shows that the variation increased considerably. This is also the expected result where a uniform and standardised medium is not used.

The question as to which of the sensitivity test media should be used remains open. Comparison of the calculated C.V. of MHA and ISA shows (Table 5) that only streptomycin, gentamycin, tetracycline, sulphafurazole and trimethoprim gave more than 1% variation on MHA when compared to ISA. Only in the case of gentamycin, tetracycline and sulphafurazole does this exceed 2% and in none of the cases is it more than 2,5%. When deciding on a routine sensitivity test medium, factors such as suitability, cost, reliability and repeatability should also be considered. MHA is used successfully worldwide in many laboratories and also has a price advantage over ISA. It is readily available in a dehydrated form.

Besides the individual variation of the various drugs used due to different test media, the results of an organisms's antibiogram should be the same when tested later under identical circumstances for the same characteristic (susceptibility or resistance). In many laboratories scant attention is paid to keeping the circumstances of the test as similar and uniform as possible. Problems of reliability are concerned with the accu-

Table 5. **Effect of the medium on the coefficient of variation (C.V.) and the average size of the zones of inhibition (x) with the test organism** *Escherichia coli* **NCTC 10418**

	MHA (n=5)			I.S.A. (n=5)			MHA + ISA (combined) (n=10)		
	$\bar{x}$ (mm)	S_{n-1}*	C.V.*	$\bar{x}$ (mm)	S_{n-1}*	C.V.*	$\bar{x}$ (mm)	S_{n-1}*	C.V.*
AP10	21,0	0,71	3,37	21,2	0,84	3,95	21,1	0,74	3,50
S10	21,4	0,89	4,18	20,2	0,45	2,21	20,8	0,92	4,42
K30	26,8	0,84	3,12	27,8	0,84	3,01	27,3	0,95	3,47
NE10	20,6	0,89	4,34	21,4	0,89	4,18	21,0	0,94	4,49
GM10	28,8	1,10	3,80	28,2	0,45	1,59	28,5	0,85	2,98
T25	21,6	1,14	5,28	18,6	0,55	2,94	20,1	1,79	8,92
C25	31,2	1,10	3,51	32,4	0,89	2,76	31,8	1,14	3,57
SF200	30,6	1,95	6,37	33,0	1,41	4,29	31,8	2,04	6,43
TM2,5	29,2	1,10	3,75	31,2	0,84	2,68	30,2	1,40	4,63
FZ200	30,0	0,71	2,30	31,0	0,71	2,28	30,5	0,85	2,79

* Actual values rounded to the second decimal digit

racy with which a test measures what it is supposed to measure[7]. Knowing its reliability, we can interpret data from the test with some known degree of certainty. With the reliability coefficient known, the standard error of measurement can be calculated. The standard error of measurement gives a more realistic indication of how uncertain the estimate of the true score is, even with relatively high reliability coefficients[7].

The true size of the zone of inhibition of an organism tested against a specific drug will be somewhere within the boundaries of ± 1,0 *Se*, with a certainty of 68%, from the size of the zone of inhibition obtained with the antibiogram. Similarly the true zone size will, with a certainty of 95%, lie within ± 1,96 *Se* of the measured zone size[7]. This information is important in the interpretation of antibiogram results where the zone sizes of bacterial isolates are correlated with the zone sizes obtained with known sensitive control cultures tested under similar conditions. It is imperative to know the reliability and reproducibility of the test especially when considering cut-off values between the categories sensitive, moderately sensitive and resistant such as those proposed by Garrod & Waterworth[5].

The method of measuring the diameter of the zone of inhibition used in this study can at best differentiate with increments of 0,5 mm. Considering this when interpreting the data in Table 6, it is evident that at the level of 95% certainty, very little difference exists between MHA and ISA with regard to the reliability of the zone sizes. When the data for MHA and ISA is combined, a slight loss in repeatability is observed. The conclusion can be made that where undefined media are used and the variation is multiple, the loss in repeatability will be even greater, especially where factors such as the depth of the test medium, and the density of the inoculum are not kept constant, as in the current study.

With all the other variables kept constant except the test organism itself, it is clear (Table 7) that at the 95% confidence level, no differences exist between *Salmonella* and *E.coli*. Both are however Enterobacteriaceae with practically the same generation time and growth rate. With a $5x10^{-3}$ dilution rate, both gave a dense but not confluent growth after 24 hours. For Gram positive cocci, a dilution factor of 10^{-3} usually gives rise to the same growth, whereas in other organisms with different growth rates, the dilution factor will have to be adapted individually[3].

Table 6. **Effect of the variable medium on the standard measurement of error of the size of the inhibition zones, with test organism *Escherichia coli* NCTC 10418**

Dilution of inoculum	Medium	n	S_{n-1}*	r_2*	Se*	
					1,0Se	1,96Se
					68%	95%
$5x10^{-3}$	MHA	50	4,37	0,94	1,05	2,06
$5x10^{-3}$	ISA	50	5,40	0,98	0,08	1,57
$5x10^{-3}$	MHA+ISA	100	4,89	0,94	1,18	2,31

* Actual values rounded to the second decimal digit

Table 7. **Effect of a variable inoculum density of the test organisms using a constant medium, on the standard measurement of error of the size of the zone inhibition**

Organisms	Dilution of inoculum	n	S_{n-1}*	r_1*	Se*	
					1,0Se	1,96Se
					68%	95%
Salmonella LT2 trp 8/N	$5x10^{-3}$	250	5,07	0,93	1,30	2,55
E. coli J62/N	a) $5x10^{-3}$	260	5,51	0,95	1,27	2,49
	b) $5x10^{-4}$	120	6,64	0,91	1,94	3,80
	a) + b) combined	380	6,29	0,80	2,81	5,51

* Actual values rounded to the second decimal digit

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KLIËNTPROFIEL VAN 'N GESELSKAPSDIERPRAKTYK

J S J ODENDAAL* en A WEYERS**

ABSTRACT:

The aim of the study was to gather demographic information from consulting clients, to compile a profile of the typical companion animal client in South Africa. This method differed from other studies in that information was collected from actual clients and not from pet owners. Completed questionnaires (n = 612) were received back from veterinary practices (n = 120) in South Africa. The data was processed by a computer. Questionnaires were completed on a voluntary basis and were anonymous.

The typical client in this survey was a young married woman, with one or two children, living in a suburban home, with an average income and 2 companion animals. The most common companion animal presented, proved to be a miniature breed of dog. The advantage of this study is that veterinarians may use it to prepare themselves in terms of the most common type of client as well as of a variety of other types. The client profile also gives an indication of the level at which consultations should be conducted, and may aid the veterinarian in developing a specific sensitivity towards certain clients.

Key words: Client profile, companion animal practice

Odendaal J.S.J.; Weyers A. **Client profile of a companion animal practice.** *Journal of the South African Veterinary Association* (1989) 60 No. 1, 25-27 (Afr) Department of Zootechnology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INLEIDING

Op die oomblik is daar geen inligting in Suid-Afrika beskikbaar oor die kliënt wat die mees waarskynlike besoeker aan die geselskapsdierveearts is nie. Die doel van hierdie studie was om demografiese inligting te versamel ten einde 'n profiel van 'n tipiese kliënt van 'n geselskapsdierpraktyk saam te kan stel. 'n Belangrike onderskeidende eienskap van hierdie studie is dat die inligting direk van konsulterende kliënte ingesamel is.

Alhoewel daar 'n aantal ondersoeke gedoen is oor verskeie aspekte van geselskapsdiereienaarskap by sekere groepe mense, kon nie een gevind word waar inligting van die werklike veeartskliënt verkry is nie. Die tipiese geselskapsdiereienaar is nie noodwendig dieselfde persoon as die tipiese veeartskliënt nie. Charles, Charles & Associates [1] verwys in hulle ondersoek oor die benutting van veterinêre dienste deur geselskapsdiereienaars, na gebruikers ("users"), en nie-gebruikers ("non-users"). Nie-gebruikers dui op geselskapsdiereienaars wat in die jaar van die ondersoek van geen veterinêre dienste gebruik gemaak het nie. Die persentasie van sulke eienaars word redelik hoog gestel: 26,4% vir honde-eienaars, 52,8% vir kateienaars en 93% vir alle ander geselskapsdiere. Verder is persone wat wel diere aanbied vir konsultasie, nie altyd die primêre eienaars nie. Hierdie feite is dus in die meeste ondersoeke uit die oog verloor.

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Lewis[2] het eenvoudig 553 telefoonnommers geskakel totdat hy 250 geselskapsdiereienaars in die metropolitaanse gebied Denver, Colorado, VSA, gevind het. Uit hierdie inligting het hy sy "Profile of clients" (dit wil sê veterinêre kliënte) gepubliseer. Salmon & Salmon[5] van Melbourne, Australië en Osterhoff[4] van Suid-Afrika, het hul inligting verkry deur gebruik te maak van vraelyste, wat ingevul is tydens deur-tot-deur besoeke. Laasgenoemde studie het slegs honde betrek. Salmon & Salmon[5] het 380 huishoudings besoek wat 1 063 mense gehuisves het. Osterhoff[4] het gebruik gemaak van veterinêre studente om die vraelyste per honde-eienaar te laat invul en meer as 600 persone is op dié wyse betrek. Charles, Charles & Associates [1] van Amerika het wel ook 'n landswye ondersoek gedoen, maar die inligting d.m.v. vraelyste per pos ingesamel. Van die 20 000 vraelyste wat aan huishoudings versend is, is 13 506 terugontvang. Uit die Charles, Charles & Associates [1] verslag het 'n verdere publikasie verskyn, wat toegelig is deur Wise[6]. Messent[3] het in Groot Brittanje 'n ondersoek gedoen wat 8 000 huishoudings betrek het. Die metode waarvolgens die vraelyste voltooi is, is nie aangedui nie.

Die voordele van hierdie studie is dat dit die veearts kan voorberei vir 'n verskeidenheid van kliënte, wat dan ook die mees algemene soort insluit. Dit werp verder ook lig op die vlakke van konsultasie wat die veearts moet kan hanteer. Agtergrondinligting oor kliënte se persoonlike besonderhede, verskaf ook insig oor spesifieke sensitiwiteite wat teenoor sekere kliënte ontwikkel moet word.

MATERIAAL EN METODE

Die vraelyste wat gebruik is in die studie is saamgestel in samewerking met die Eenheid vir Professionele Opleiding en Dienslewering in die Gedragswetenskappe (EPOG) aan die Universiteit van die Oranje-Vrystaat (UOVS).

In 'n landswye ondersoek is vraelyste (n = 1 200) aan veeartse (n = 120) regoor Suid-Afrika gestuur. Die praktyke is geselekteer op grond daarvan dat hulle oorwegend geselskapsdiereienaars bedien. Slegs vrywillige kliënte wat die veearts by die kliniek/hospitaal besoek het vir konsultasies, is betrek by die voltooiing van die vraelyste. Die vraelyste is anoniem voltooi en terugbesorg aan EPOG. Ses-honderd-en-twaalf voltooide vraelyste is terugontvang en dien dus as 'n steekproef van konsulterende veeartskliënte in Suid-Afrika. Die antwoorde is deur die Buro vir Rekenaardienste aan die UOVS verwerk.

RESULTATE

Die resultate het voorsiening gemaak vir 'n kolom wat as "Onbekend" aangedui word, as kliënte vrae nie kon of wou beantwoord nie. Slegs 1-6% (gemiddeld 2%) van die totale aantal antwoorde het in die kolom "Onbekend" beland (Tabel 1-11).

BESPREKING

Geslag

Hierdie ondersoek het gevind dat die meerderheid van veeartskliënte vroulik is. Die tendens is ook in ooreenstemming met dié van ander studies[1 4 5].

Tabel 1: **Geslag van veeartskliënte van 'n geselskapsdierpraktyk**

Manlik	39%
Vroulik	61%

Ouderdom

Die versorging van geselskapsdiere, ruimte, fisiese en geestelike krag en finansiële oorwegings mag beperkend inwerk op die aanhou van geselskapsdiere deur bejaardes (Tabel 2). Die veearts kan nietemin 'n betekenisvolle ondersteunende bydrae lewer aan die 5% kliënte van 60 jaar en ouer, omdat geselskapsdiere feitlik sonder uitsondering vir elkeen van daardie individue baie belangrik is. Die neiging dat bejaardes 'n kleiner groep van geselskapsdiereienaars vorm, is in ooreenstemming met resultate verkry in ander opnames[1-3 5 6].

Charles, Charles & Associates[1] het beweer dat manlikes onder die ouderdom van 18 jaar in net 5% van huishoudings vir die versorging van honde verantwoordelik is en vroulikes onder 18 slegs in 4% huishoudings. In slegs 2% van die huishoudings met honde, neem kinders besluite wanneer die hond die veearts moet besoek. Osterhoff[4] het gevind dat 21% van kinders in huishoudings honde versorg en dat in 30% van gevalle, honde die meeste aan die kinders geheg is. In 42% van gevalle was die honde die meeste aan die moeder in die huis geheg.

Salmon & Salmon[5] het gevind dat in 12% van huishoudings die kind as eienaar beskou word, terwyl in 49% van huishoudings die geselskapsdier as die eiendom van die hele gesin beskou word. Op 'n vraag wie

die dier veearts toe neem, het 6% aangedui dat dit die kinders is.

In hierdie studie was 8% veeartskliënte onder die ouderdom van 20 jaar, wat baie min met die studie van Salmon & Salmon[5] ooreenstem. Dit bewys weer eens dat indien die vrae direk betrekking op kliënte het eerder as op eienaarskap, daar meer betroubare resultate verkry kan word. Die belangrikheid van kinders wat die veearts besoek moet nooit onderskat word nie. As negatiewe indrukke van die veearts gedurende die kinderjare opgedoen word, kan dit nie net besoeke aan die veearts beïnvloed nie, maar inderdaad ook eienaarskap.

Tabel 2: **Ouderdom van veeartskliënte van 'n geselskapsdierpraktyk**

0- 9 jaar	2%
10-19 jaar	6%
20-29 jaar	25%
30-39 jaar	30%
40-49 jaar	21%
50-59 jaar	11%
60-69 jaar	4%
70-79 jaar	1%
80 jaar en ouer	0%

Opvoedkundige kwalifikasies

Die onvermoë van 'n veearts om op die kliënt se vlak te kommunikeer kan baie gou veearts-kliënt-verhoudings versteur. Die 18% kliënte wat 'n Universiteitsgraad of hoër kwalifikasies het, moet as besonder hoog beskou word, as die persentasie persone wat grade in die totale bevolkingsopset besit in ag geneem word. Volgens die Sentrale Statistiekdiens was die gegradueerdes in Suid-Afrika in 1985 slegs 5%. Die resultate in hierdie studie kan dus 'n toevallige bevinding wees of dalk 'n werklike aanduiding van die mate waarin die intelligensia van veterinêre dienste gebruik maak.

Tabel 3: **Hoogste opvoedkundige kwalifikasie van veeartskliënte van 'n geselskapsdierpraktyk**

Geen	0%
Primêre skool	3%
Sekondêre skool	20%
Standerd 10/Sertifikaat/Diploma	48%
Universiteitsgraad	18%
Nagraadse studie	11%

Huwelikstaat

Ander bevindings[1,2,5] stem baie met hierdie studie se syfers ooreen, wat aantoon dat ongeveer 80% van die mense wat die veearts besoek, getroud is. Hierdie studie het egter vasgestel dat 4% kliënte geskei en 17% alleenlopers is. Diere beteken vir alleenlopers dikwels meer as vir die lede van 'n gemiddelde huisgesin. Hierdie groep plaas dan ook dikwels 'n baie groter emosionele las op die veearts as ander groepe.

Tabel 4: **Huwelikstaat van veeartskliënte van 'n geselskapsdierpraktyk**

Getroud	79%
Ongetroud	17%
Geskei	4%

Inkomste per jaar

Inkomste per jaar is uiteraard baie moeilik om tussen lande, sowel as tussen jare te vergelyk. Wat hier van groter waarde is, is die verspreiding van inkomste onder eienaars van veeartskliënte.

Die verspreiding van inkomste van veeartskliënte in hierdie studie bevestig soos in die ander studies[1-4] dat daar 'n redelike eweredige verspreiding van inkomste onder veeartskliënte, sowel as geselskapsdiereienaars bestaan. Indien die kategorieë ietwat anders saamgestel word, kan daar selfs 'n meer eweredige verspreiding verkry word. Daar is egter tog 'n neiging dat die gemiddelde en hoër inkomste groepe meer dikwels as veeartskliënte geïdentifiseer word. Dit korreleer dalk met die persentasie hoër opvoedkundige kwalifikasies. By die vasstelling van die professionele foole vir veterinêre dienste, sal hierdie eweredige verspreiding van inkomstes onder die kliënte ook oorweeg moet word.

Tabel 5: Huidige inkomste per jaar (1985) vir veeartskliënte van 'n geselskapsdierpraktyk

R 0-R 4 999	16%
R 5 000-R 9 999	15%
R10 000-R19 000	31%
R20 000-R29 999	17%
R30 000 en meer	21%

Ras

Hierdie studie se hoë blankesyfer van 97% teenoor die 3% anderskleuriges kan verklaar word aan die hand van die feit dat voorstedelike geselskapsdierpraktyke nie geredelik bereikbaar is vir anderskleuriges wat meesal ver van hierdie praktyke bly nie. Ander faktore mag ook 'n rol speel, maar dit blyk dat die anderskleurige kliënte wat wel die moeite doen om sulke praktyke te besoek, min of geen verskille toon met ander kliënte. Die opleiding van anderskleurige veeartse wat op die oomblik in volle swang is, sal na verwagting die deelname van anderskleuriges in geselskapsdierpraktyk, drasties laat toeneem.

Tabel 6: **Ras van veeartskliënte van 'n geselskapsdierpraktyk**

Blank	97%
Gekleurd	2%
Swart	1%

Kinders per gesin

Dit lyk nie asof die grootte van 'n gesin enige korrelasie met die aanhou van diere, óf besoeke aan die veearts, toon nie, maar wel met die algemene neiging in die moderne gemeenskappe om kleiner huisgesinne te hê.

Twee ander studies, een in die VSA[1] en een in Suid-Afrika[4], het ook aangetoon dat geselskapsdiereienaars in die meeste gevalle twee kinders per gesin het. Salmon & Salmon[5] het bevind dat 65% van geselskapsdiereienaars, kinders het.

Tabel 7: **Aantal kinders per gesin van veeartskliënte van 'n geselskapsdierpraktyk**

Nie van toepassing of geen	38%
Een	21%
Twee	25%
Drie	11%
Vier en meer	5%

Woongebied

Salmon & Salmon[5] het slegs 'n onderskeid gemaak tussen die ligging van geselskapsdiereienaars se woonplekke in die stad en dit verdeel in binne-voorstedelike (8%), middel-voorstedelike (65%) en buite-voorstedelike (20%) gebiede. Die 27% wat nie hierby ingepas het nie, se detail is onbekend.

In hierdie studie kon 'n baie belangrike feit vasgestel word, deurdat bepaal kon word hoeveel plattelanders wel "gespesialiseerde" geselskapsdierpraktyke besoek. Geen ander studie het hierdie verskynsel ondersoek nie en die 17% in hierdie studie moet as relatief hoog beskou word. Dit onderskryf die mening dat 'n beduidende persentasie plattelanders ook bereid is om veeartskostes ten opsigte van hul geselskapsdiere aan te gaan. Verder dui dit daarop dat geselskapsdiere vir hierdie eienaars 'n sentimentele waarde het, wat meesal gepaard gaan met emosionele binding tussen eienaar en dier.

Tabel 8: **Woongebied vir veeartskliënte van 'n geselskapsdierpraktyk**

Stad	83%
Platteland	17%

Woonplek

Hierdie studie bevind dat 79% van veeartskliënte in huise woon, terwyl 9% vanaf plase kom. As die laaste syfer in verband met die vorige vraag gesien word, beteken dit dat 8% vanaf plattelandse dorpe kom. In stede lyk dit tog of beskikbare ruimte weer eens 'n belangrike rol kan speel in die aanhou van geselskapsdiere, as daarop gelet word dat daar slegs 12% kliënte is wat in kleiner wooneenhede bly.

Tabel 9: **Woonplek van veeartskliënte van 'n geselskapsdierpraktyk**

Huis	79%
Woonstel/Meenthuis	12%
Plaas	9%

Tipe dier

Hierdie studie het getoon dat 61% van die veeartskliënte honde-eienaars was en 21% kateienaars. Die kliënte met katte is dus relatief baie, sowel as die 12% wat voëls aanhou. 'n Mens moet in ag neem dat daar ook multi-spesie-eienaarskap kan voorkom en van die ander studies[1,2,7] het hierdie verskynsel uitgewys. Streng regulasies oor die aanhou van eksotiese diere is moontlik deels verantwoordelik vir die 6% "ander diere". Onder hierdie ander diere is rotte, muise en hamsters seker die gewildste.

Tabel 10: **Tipe geselskapsdier van veeartskliënte van 'n geselskapsdierpraktyk**

Klein hond	33%
Groot hond	28%
Kat	21%
Voël	12%
Ander	6%

Diere per gesin

In hierdie studie is gevind dat die meeste kliënte twee diere per gesin aanhou (30%) en dat 68% een tot drie diere aanhou. Die betekenis van hierdie inligting vir die veearts is dat, as hy een kliënt verloor, hy terselfdertyd meer as een pasiënt verloor. In totaal gesien, kan 'n mens daarop wys dat 81% van kliënte meer as een dier aanhou.

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Die meer as 16% wat meer as ses diere aanhou, sal veral gevind word onder voëlboere, honde- en kattelers en kateiénaars. Verder kan die aanhou van 'n verskeidenheid geselskapsdiere ook die aantal per eienaar opstoot, aangesien baie van hierdie diertjies van die kleiner soort is.

Tabel 11: **Aantal diere per gesin van veeartskliënte van 'n geselskapsdierpraktyk**

Een	19%
Twee	30%
Drie	19%
Vier	8%
Vyf	8%
Ses of meer	16%

Dit is duidelik dat hierdie studie meer insig aan veeartse kan gee oor hul kliënte en dat hierdie insig wel hulle houding en benadering tot hul praktyk kan beïnvloed. Uit hierdie resultate kan daar dan 'n profiel van die tipiese geselskapsdierkliënt getrek word.

Die profiel lyk soos volg:

Geslag:	Vroulik
Ouderdom:	35 jaar
Kwalifikasie:	St 10 Diploma of Sertifikaat
Huwelikstaat:	Getroud
Inkomste	R15 000 per jaar
Ras:	Blank
Kinders:	Twee
Woongebied:	Stedelik
Woonplek:	Huis
Geselskapsdier	Hond (klein)
Aantal diere	Twee

Hierdie profiel pas dus in by die gemiddelde werkende, redelik jong getroude, blanke vrou met twee kinders, wat in 'n voorstedelike huis woon en twee geselskapsdiere aanhou. Hierdie beeld pas baie goed in by die area waarin die meeste voorstedelike geselskapsdierpraktyke in Suid-Afrika huidig geleë is.

Dit sou egter 'n ernstige fout wees om slegs vir die tipiese kliënt in die praktyk voorsiening te maak. Die uitsonderings en variasies het dikwels die veearts, as 'n professionele persoon, méér nodig as die gemiddelde kliënt. Al sou 'n spesifieke uitsondering van 'n soort kliënt slegs 5% in 'n praktyk voorkom, kan dit 'n betekenisvolle impak op die praktyk hê. Veronderstel 'n veearts konsulteer 12 000 kliënte per jaar en 5% kliënte toon 'n hoë emosionele eienskap, dan beteken dit dat die veearts 600 moeilike konsultasies van daardie spesifieke kliënte, per jaar gaan behartig. Op 'n maandelikse basis is dit 50 konsultasies en op 'n dag-basis byna twee per dag vir elke dag van die jaar. Selfs teen die helfte minder konsultasies, is dit byna een so 'n soort konsultasie per dag.

Die doel van hierdie studie is dus nie net om die tipiese veeartskliënt se profiel vas te stel nie, maar om óók kennis te neem van die voorkoms van die soort kliënte wat buite die tipiese profiel val.

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THE INFLUENCE OF EXPERIMENTALLY INDUCED COPPER DEFICIENCY ON THE FERTILITY OF RAMS. I. SEMEN PARAMETERS AND PERIPHERAL PLASMA ANDROGEN CONCENTRATION

F E VAN NIEKERK* and C H VAN NIEKERK**

ABSTRACT:

The influence of molybdenum and molybdenum plus sulphate-induced copper deficiencies on semen quality and peripheral plasma testosterone concentrations in South African Mutton Merino rams was investigated. All animals received an identical ration, supplemented with molybdenum in one group (M) and molybdenum plus sulphate in another (MS) while the control group (C) received additional copper supplementation. After almost a year on these rations, rams in Group MS produced ejaculates of lower volume, lower sperm concentration, poorer sperm motility and morphology than rams of the other 2 groups. The fructose concentration in the ejaculates of group MS rams was also lower than that of rams in the other groups. Peripheral plasma testosterone concentrations in Group MS were lower than in Groups C or M. Liver copper concentrations and clinical signs were consistent with a severe copper deficiency in Group MS and a mild deficiency in Group M. After the copper deficiency was reversed, the above parameters reverted to normal. It was concluded that an experimentally induced copper deficiency produced reversible impairment of testicular function in rams.

Key words: Rams, induced copper deficiency, semen quality, testosterone production

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INTRODUCTION

Increased conception rates in ewes mated to rams with high plasma copper concentrations compared to rams with low plasma copper concentrations have been reported by Weiner et al.[15]. Due to the fact that semen evaluations were not performed on these rams, the specific reasons for the reported difference in conception rates are not known. By supplementing the food of bulls with copper, Jercovic[6] succeeded in improving the semen quality of these animals as shown by the reduced occurrence of dead sperm. Thomas & Moss[11] have reported a total lack of libido in young bulls which were subjected to molybdenum supplementation in their diet.

Although these reports suggest that a copper deficiency influences the reproductive processes in the male, the precise physiological and biochemical functions which are affected are not known at present. The aim of this study has been to determine whether a molybdenum-sulphate induced copper deficiency would influence the semen quality and plasma testosterone concentrations of rams.

MATERIALS AND METHODS

Animals, treatment and procedure

Thirty-six 8-month-old S.A. Mutton Merino ram lambs were divided into a control (C) and 2 treatment groups (M and MS) in order to induce a copper deficiency in them. The 36 rams were divided so that the 3 groups comprised an equal number of animals of haemoglobin types AA, AB and BB. The average body mass of the 3 groups were (C) 32,8; (M) 33,3 and (MS) 31,6 kg. The diets of the 3 groups were basically the same and consisted of 45,3% oat hay, 38% oats, 10% lucerne hay, 1,5% feed grade urea, 2,5% white sugar, 1,0% $CaCo_3$, 0,3% Na_2Po_4 and 0,7% NaCl. The ration of the control group (C) was supplemented with 15g $CuSO_4$ per ton of feed; those for groups M and MS with 100 g $(NH_4)_6 Mo_4.4H_2O$ per ton and that for group MS additionally, with 4,5 kg Na_2So_4 per ton as described by Van Niekerk & Van Niekerk[13]. During the experimental period the animals were housed individually in a feeding shed and received 1,5 kg of the respective diets on a daily basis. After the animals had received the respective diets for a period of 4 months, 3 rams of each group were slaughtered and liver and kidney samples collected for trace mineral analyses[13]. One ram in group MS was injured and had to be destroyed.

After a feeding period of 12 months, semen samples were collected from 6 rams, (2 of each haemoglobin type, in each group). Semen samples were collected once a week for 4 consecutive weeks from the same rams by means of electro-ejaculation[1]. These semen samples were the first ever collected from these rams which had thus far never been used for natural mating. The first and last semen samples that were collected from each of the 18 rams in the 3 treatment groups were also subjected to a thorough bacteriological examination. Collections were done on each occasion by the same operator. Blood samples (20 ml) were collected from the jugular vein in heparinised vacuum tubes after each semen collection. Ten ml of whole blood was kept for selenium determinations while the remaining 10 ml was centrifuged at 3 000 r.p.m. and the plasma stored at -20°C until it was analysed for copper and zinc.

At the end of the 4 week semen-collection period, 5 ml blood samples were taken in heparinised tubes on an hourly basis for a 24 hour period from 3 rams in each group. These rams were selected from the rams of which semen had been collected and there was one of each haemoglobin type. The samples were immediately centrifuged at 3 000 r.p.m. and the plasma removed and stored at -20°C until it was analysed for plasma testosterone concentrations. At this stage of the experiment the remaining 3 rams from which semen was collected, were slaughtered and liver and kidney samples taken for trace mineral analyses[13].

Of the remaining 5 rams in group MS, 2 were put on the control diet (C) while the other 3 remained on diet MS. Two of the latter 3 rams were dosed with 10 mg Se as sodium selenite in an aqueous solution. This was repeated after 30 d. Semen samples of these 5 rams were collected by means of electro-ejaculation[1] after they had been on the revised rations for 70 d.

Analytical methods

Blood was analysed for selenium while plasma was analysed for copper and zinc. Liver and kidney samples were analysed for copper, zinc, iron, manganese, selenium and molybdenum using described methods [14].

The total plasma testosterone and dihidrotestosterone (DHT) concentration was determined by radio-immuno-assay using a commercial kit (Radiochemical Centre, Amersham, England; TRK 600). The maximum value during a gradual rise and decline in plasma testosterone concentrations was defined as a peak value.

Semen was macroscopically evaluated for consistency and volume and the pH determined with pH paper (range 5,4-8,0; Merck Chemicals). Semen volume was recorded directly from the graduated collection tubes. Ejaculates were evaluated microscopically for sperm motility[15], the percentage live sperm (nigrosin + eosin method) as well as morphological abnormalities as described by Campbell et al.[2]. Semen smears were also stained according to the Karras method for morphological evaluation[13]. The sperm concentration was determinea with a haemocytometer and in duplicate samples by means of an automatic "Semen concentration photometer" (IMV, France). The fructose con-

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tent of the ejaculates was determined according to the method described by Mann[7]. Haemoglobin typing was done by the blood typing laboratory of the ADSRI by means of gel-electrophoresis[14].

Statistical analyses

The results were analysed according to standard one way analysis of variance procedures, using the P1V programme of the BMDP statistical packet[3]. Differences between treatment means were tested by Student's *t*-test[10].

RESULTS

Blood and liver trace mineral concentrations

The mean blood selenium and plasma copper and zinc concentrations of the rams during the semen collection period are shown in Table 1. The liver trace mineral concentrations of the rams after completion of the semen collection period are shown in Table 2.

Macroscopic evaluation of semen

The mean semen volumes (Table 3) of the ejaculates of the rams in groups C and M were normal and did not differ significantly. The semen volume of the rams in group MS, which suffered from a severe copper deficiency, was much lower at all samplings in comparison with the semen volume of groups C and M.

The consistency of the semen of rams from group C varied from thick creamy to creamy. In group M it varied from thick creamy to milky and in group MS nearly all the samples had a watery appearance.

The average pH values (Table 3) for the different semen collections in all 3 groups varied within the normal limits of 6,4 to 7,2[5]. Treatment had no significant influence on the pH of the semen.

Microscopic evaluation of semen

Sperm motility in samples obtained from rams in group C varied between 4 and 5 which implies that 65 to 100 % of the sperm showed progressive movement[15]. In the case of group M, motility varied between 3 and 5, meaning that 45 to 100 % of the sperm showed progressive movement. Sperm motility in the case of group MS was minimal (0-1) which implies that 0 to 20 % of the sperm showed movement.

The mean sperm concentration (Table 4), as determined by both methods, varied between 1 280 and 2 200 million sperm/ml in group C.

In group M there was a 50% decrease in the sperm concentration between the first and fourth samplings. In group MS sperm count was severely suppressed.

The percentage live sperm (Table 3) in the collected semen from rams in group C varied between 90 and 96, in group M between 85 and 95 while in group MS only 25-70% live sperm were found.

The percentage abnormal sperm (Table 3) found in the semen samples obtained from group C varied between 1 and 4. Loose sperm heads were virtually the only sperm abnormality found in group C. Abnormalities in semen samples from group M varied between 2 and 11% and consisted mostly of loose sperm heads and curled tails. The percentage abnormal sperm in group MS varied from 45 to 90%. The different types of abnormalities that contributed to the total percentage abnormalities in this group are given in Table 4.

Fructose concentration

The average initial fructose concentration (Table 5) in semen samples at the time of collection (time 0) did not differ between rams in groups C and M, but the fructose concentration in semen samples from rams in group MS was approximately 40% lower.

Plasma testosterone concentrations

The number of testosterone peaks and the maximum concentrations thereof are summarised in Table 6.

On average the number of testosterone peaks per 24 h periods was the same for the 3 groups. Although it is evident that there was a significant variation in testosterone concentrations between rams in the same group, it is nevertheless clear that the production of testosterone in group MS was severely suppressed.

Mineral supplementation of rams in group MS

Seventy d after the rams received the mineral supplementation, semen samples were collected and evaluated; the results of which are summarised in Table 7.

It is evident that when the copper deficiency was reversed spermatogenesis resumed.

All bacteriological examinations of semen were negative.

DISCUSSION

The liver copper concentrations of the rams (Table 2) indicated that animals in group M suffered a mild copper deficiency and animals in group MS from a severe deficiency, while the copper concentrations in group C (control) were normal. Liver copper concentrations of less than 50 μg Cu/g DM are regarded as severely deficient while liver copper concentrations of 50 to 100 μ Cu/g DM are regarded as sufficient (unpublished results). The reliability of the total plasma copper concentrations as seen from a diagnostic point of view has been discussed[14]. Liver zinc, manganese, iron and selenium concentrations (Table 2)

Table 1: **The mean (± SD) blood selenium, plasma copper and plasma zinc concentrations of the rams in the 3 groups during the semen collection period**

Mineral	Treatment group	Time in weeks 1	2	3	4
Selenium (mmol ℓ⁻¹)	C	0,81 ± 0,13	0,86 ± 0,14	0,88 ± 0,17	0,86 ± 0,10
	M	0,79 ± 0,06	0,79 ± 0,06	0,86 ± 0,05	0,91 ± 0,05
	MS	0,64 ± 0,07	0,68 ± 0,10	0,67 ± 0,10	0,67 ± 0,08
Copper (mmol ℓ⁻¹)	C	14,7 ± 2,9	15,2 ± 2,8	16,8 ± 2,9	20,4 ± 3,9
	M	20,1 ± 4,8	20,7 ± 3,3	20,6 ± 3,1	19,8 ± 3,9
	MS	13,5 ± 4,2	12,2 ± 4,4	12,4 ± 3,7	12,1 ± 4,2
Zinc (mmol ℓ⁻¹)	C	11,1 ± 0,4	11,3 ± 1,3	11,6 ± 1,2	11,5 ± 1,5
	M	11,9 ± 1,2	11,5 ± 1,6	11,9 ± 0,9	12,8 ± 0,9
	MS	13,0 ± 1,3	12,6 ± 1,2	12,1 ± 1,8	12,5 ± 1,1

Group C — Control group
Group M — Molybdenum supplemented group
Group MS — Molybdenum and sulphate supplemented group

Table 2: **The mean (± SD) liver trace element concentration of the rams in the 3 groups at the end of the semen collection period**

Treatment group	Trace element concentration (μg g⁻¹) Cu	Zn	Mn	Mo	Fe	Se
C	256 ± 57	109 ± 33	12 ± 1,5	4,0 ± 1,5	487 ± 117	0,44 ± 0,04
M	67 ± 21	116 ± 30	10 ± 2,0	16,9 ± 9,7	494 ± 97	0,43 ± 0,01
MS	32 ± 28	91 ± 32	6 ± 0,5	11,7 ± 5,2	463 ± 150	0,42 ± 0,02

Table 3: **The mean sperm concentration (± SD), the percentage live (± SD) and abnormal (± SD) sperm and the volume and pH of the ejaculates of the rams in the 3 groups**

Group	Ejaculate no	Sperm			Ejaculate	
		Concentration per ml (x 10^6)	% Live	% Abnormal	Volume (ml)	pH
C	1	1618 ± 335	92 ± 2,6	2,0 ± 0,9	1,4 ± 0,3	6,88 ± 0,12
		(1280 - 2200)	(90 - 95)	(1 - 3)	(1,0 - 1,8)	(6,7 - 7,0)
	2	1431 ± 281	93 ± 2,4	2,7 ± 0,5	1,2 ± 0,5	6,78 ± 0,17
		(1100 - 1830)	(90 - 96)	(2 - 3)	(1,0 - 1,4)	(6,6 - 7,0)
	3	1510 ± 320	93 ± 2,0	2,2 ± 0,8	1,4 ± 0,4	6,83 ± 0,05
		(1170 - 2200)	(90 - 95)	(1 - 3)	(1,0 - 1,4	(6,8 - 6,9)
	4	1480 ± 329	93 ± 2,7	2,7 ± 1,0	1,2 ± 0,2	6,83 ± 0,10
		(1000 - 1850)	(90 - 96)	(1 - 4)	(0,9 - 1,5)	(6,7 - 7,0)
M	1	1701 ± 415	90 ± 2,9	5,7 ± 1,8	1,3 ± 0,3	6,83 ± 0,14
		(1080 - 2150)	(85 - 92)	(3 - 8)	(1,1 - 1,8)	(6,6 - 7,0)
	2	1098 ± 228	(91 - 2,5)	7,8 ± 2,0	1,2 ± 0,4	6,75 ± 0,1
		(680 - 1310)	(88 - 92)	(5 - 11)	(0,8 - 2,0)	(6,6 - 6,8)
	3	626 ± 268	91 ± 1,6	5,2 ± 3,0	1,3 ± 0,2	6,81 ± 0,13
		(300 - 1010)	(88 - 92)	(2 - 9)	(0,9 - 1,6)	(6,6 - 6,9)
	4	725 ± 154	91 ± 2,5	6,5 ± 1,4	1,3 ± 0,2	6,77 ± 0,22
		(500 - 900)	(88 - 95)	(5 - 9)	(61,1 - 1,6)	(6,5 - 7,0)
MS	1	150 ± 71	50 ± 8,2	65,6 ± 15,6	0,8 ± 0,3	6,80 ± 0,09
		(90 - 260)	(45 - 62)	(45 - 90)	(0,5 - 1,2)	(6,7 - 6,9)
	2	7,3 ± 2,1	57 ± 90	77,2 ± 6,9	0,7 ± 0,2	6,70 ± 0,39
		(6 - 10)	(45 - 65)	(65 - 85)	(0,6 - 1,0)	(6,2 - 7,0)
	3	4,6 ± 2,4	59 ± 7,5	77,5 ± 7,6	0,7 ± 0,2	6,65 ± 0,23
		(2 - 8)	(49 - 70)	(69 - 90)	(0,5 - 0,9)	(6,4 - 7,0)
	4	4,1 ± 2,2	47 ± 12,5	75,7 ± 12,1	0,8 ± 0,2	6,58 ± 0,29
		(1 - 7)	(25 - 60)	(58 - 90)	(0,6 - 1,1)	(6,3 - 6,9)

Table 4: **Sperm abnormalities (± SD) in ejaculates of group MS**

Abnormality	Range (%)
Protoplasmic droplets	(1 — 4)
Acrosome defects	(6 — 12)
Loose heads	(18 — 25)
Curled tails (loop forming)	(18 — 25)
Tail and head still connected but, the tail broken	(19 — 24)
Small malformed heads with normal tails	(6 — 12)
Sperm with underdeveloped (small) tails	(7 — 12)

were not affected by the treatment. As expected, animals in groups M and MS had abnormally high liver molybdenum concentrations if the norm of 2-4 μg Mo/g DM is taken into account[4]. According to the average blood selenium concentrations, none of the rams suffered from a selenium deficiency, since values in excess of 0,633 μmol ℓ^{-1} are regarded as sufficient[12]. The plasma zinc concentrations were normal in all three groups[12].

Semen volume (Table 3) was suppressed by a severe copper deficiency. The slightly lower pH of the semen of the rams in group MS might have resulted from the lower semen volume (Table 3), caused by a diminished contribution by the accessory sex glands which are responsible for the maintenance of semen pH[5]. Sperm motility was severly suppressed in group MS.

In group C the sperm concentration varied between the normal limits of 1 000 and 3 000 million sperm per ml[5]. Total sperm per ejaculate in group M was influenced by the mild copper deficiency and severely suppressed in group MS.

It is clear that a copper deficiency not only decreased sperm production but also increased sperm abnormalities. The large number and type of sperm abnormalities, especially morphological abnormalities such as small heads, acrosomal defects and underdeveloped and curled tails, indicate that most of these abnormalities must have developed during the process of spermiogenesis when morphological changes of the secondary spermatocytes, spermatids and spermatozoa occur. The low rate of fructolysis recorded in group MS was caused by the low sperm concentration, as well as the high percentage of dead sperms found in semen samples of this group. It is further known that testosterone plays an important role in the production of fructose by the accessory sex glands[8].

Changes observed in the plasma testosterone concentrations in groups C and M corresponded well with those reported by Purvis et al.[9]. However low plasma testosterone concentrations (Table 7) were recorded in rams of group MS. This finding certainly contributed to the suppressed fructose and sperm production observed in this group due to the well-known action of testosterone in this regard[5].

It is clear that an induced copper deficiency existing for a period of 20 months does not cause permanent damage to testicular tissue. Sperm production resumed within 70 d after the rams has been given adequate copper which indicates that the effect of a copper deficiency on the testes is reversible.

According to the liver copper concentration and the general appearance of the wool, the rams in group M suffered from a mild and rams in group MS from a severe copper deficiency. The decrease in semen volume, sperm and fructose concentration, testosterone production as well as the increase in the percentage dead and abnormal sperm in the ejaculates of group M and to a larger extent in group MS, can be ascribed to the induced copper deficiency. The extent to which these parameters were affected was related to the degree of the copper deficiency.

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Table 5: **Fructolysis as a measurement of sperm activity, as determined by the semen fructose concentration (mmol ℓ^{-1}) in two ejaculates of the rams in the 3 treatment groups**

Treatment group	Ejaculate no.	Average (±SD) sperm concentration per ml ($x10^6$)	Fructose (±SD) concentration (mmol ℓ^{-1}) during incubation (minutes)			
			0	60	120	180
C	2	1398±335	1242±222	702±187	453±128	234±26
	3	1570±350	1420±404	624±351	262±149	126±76
M	2	1098±228	1195±85	643±177	510±170	384±100
	3	475±265	1423±424	526±314	275±162	143±94
MS	2	42±43	778±374	632±346	569±338	490±310
	3	4,6±2,4	642±307	569±332	548±330	508±318

Table 6: **The number of testosterone peaks and the maximum and minimum testosterone concentrations during a 24 h period of the rams in the 3 treatment groups.**

Treatment group	Ram no	Hb type	Peaks per 24 h	Testosterone concentration (nmol ℓ^{-1})	
				mean (±SD) peak concentration (Range)	minimum
C	1	AA	5	7,91 ± 3,69 (4,69 - 14,14)	0,84
	2	AB	3	7,06 ± 2,49 (4,22 - 8,19)	0,19
	3	BB	4	10,21 ± 4,77 (4,87 - 14,25)	0,19
M	4	AA	4	4,11 ± 0,96 (2,81 - 5,25)	0,19
	5	AB	5	6,45 ± 2,04 (4,68 - 10,31)	0,66
	6	BB	5	7,51 ± 4,84 (3,56 - 14,25)	0,84
MS	5	AA	5	6,49 ± 3,14 (3,37 - 10,25)	0,28
	4	AB	4	2,86 ± 1,35 (1,97 ± 4,87)	0,19
3	BB	3	3	2,24 ± 1,46 (1,13 - 3,90)	0,19

Table 7: **Semen characteristics of rams in group MS which received mineral supplementation**

Supplementation	Semen Characteristics	Day supplementation commenced	70 d after supplementation
Received control diet	Consistency	Watery	Creamy
	% Abnormal	60	7
	% Alive	52	90
	Concentration ($x\ 10^6$/ml)	4	600
Received diet MS with Se supplementation	Consistency	Watery	Watery
	% Abnormal	63	68
	% Alive	45	47
	Concentration ($x\ 10^6$/ml)	3,8	4,0
Received diet MS	Consistency	Watery	Watery
	% Abnormal	58	62
	% Alive	39	41
	Concentration ($x\ 10^6$/ml)	4	3,5

THE INFLUENCE OF EXPERIMENTALLY INDUCED COPPER DEFICIENCY ON THE FERTILITY OF RAMS. II. MACRO- AND MICROSCOPIC CHANGES IN THE TESTES

F E VAN NIEKERK* and C H VAN NIEKERK**

ABSTRACT

The macro- and microscopic changes of the testes caused by molybdenum as well as a molybdenum plus sulphate induced copper deficiency were investigated in S.A. Mutton Merino rams. Judged on testes measurements, the testes development of rams suffering from a severe copper deficiency was slower ($P \leq 0,05$) when compared with rams in the control group. Histological examinations of the testes of the rams which suffered from a severe copper deficiency revealed that the seminiferous tubules were less developed and less active than those of the control group. This was mainly due to the inactivity of the Sertoli cells. Where rams suffered from a copper deficiency, the Sertoli cells contained only a small volume of cytoplasm. The typical fingerlike cytoplasmic evaginations of the Sertoli cells into the lumen of the seminiferous tubules were absent while the nuclei of these cells were darkly stained, in some cases even pycnotic. Spermatocytogenesis was normal because primary spermatocytes with dark stained thread-like chromatin were observed in most of the seminiferous tubules. The process of spermiogenesis (metamorphic phase) did not take place. This can be accounted for by the inactivity of the Sertoli cells.

Key words: Rams, induced copper deficiency, testes growth, testes histology

Van Niekerk F.E.; Van Niekerk C.H. The influence of experimentally induced copper deficiency on the fertility of rams. II. Macro- and microscopic changes in the testes. *Journal of the South African Veterinary Association* (1989) 60 No. 1, 32-35 (En.) Department of Human and Animal Physiology, University of Stellenbosch, 7600, Stellenbosch, Republic of South Africa.

INTRODUCTION

The process of spermatogenesis which takes place in the seminiferous tubules can be divided into two stages, namely spermatocytogenesis and spermiogenesis[6]. Both these stages are dependent on the stimulating effects of the sex hormones testosterone, follicle stimulating hormone (FSH) and luteinising hormone (LH). The second stage, spermiogenesis, also depends on the active functioning of the Sertoli cells[2]. The effect of a copper deficiency on semen production and semen quality was described by Van Niekerk & Van Niekerk[17]. They found a high percentage of morphologically abnormal spermatozoa in semen from rams which suffered from a copper deficiency. This indicates that a copper deficiency probably affects spermiogenesis. In a histological examination of the testes of 2 bull calves which received additional molybdenum in their diets for a period of 129 days, Thomas & Moss [15] found a total absence of spermatozoa and spermatids in the seminiferous tubules, with the interstitial cells showing signs of degeneration. It was not clear to what extent molybdenum supplementation influenced the body copper reserves, since the copper concentrations in neither the body nor in the rations were determined[15].

The object of this study was to investigate the effects of an induced copper deficiency on the different stages of spermatogenesis.

MATERIAL AND METHODS

Experimental animals and procedures

Eight-month-old S.A. Mutton Merino ram lambs (n = 36) were divided equally into three treatment groups. The three treatment groups received the same basic diet to which was added in the control diet (C) additional copper, in the molybdenum supplemented diet (M) additional molybdenum and in the molybdenum and sulphate supplemented diet (MS), additional molybdenum and sulphate, as described by Van Niekerk & Van Niekerk[16].

Three rams of each treatment group were slaughtered at 12 months of age and 3 more of each group at 20 months of age. Immediately after the animals had been slaughtered, both testes were removed and weighed after which a tissue sample for histological examination was taken from the middle of the right testis of each animal. It was placed in a fixative consisting of 900 ml 0,9% isotonic salt solution and 100 ml 37-40% formalin. The in situ testes' length and diameter were measured with a calibrated tape to record the size of the testes.

Analytical procedures

Testes samples were fixed, processed, imbedded, cut, stained and mounted according to standard histological procedures[8].

The slides were examined with a light microscope to compare the testis development and activity of the differentiating cells in the seminiferous tubules of the three treatment groups. The following parameters were recorded and evaluated: the number of seminiferous tubules per microscopic field; the average diameter of the seminiferous tubules; the average thickness of the epithelium of the seminiferous tubules; estimation of the percentage seminiferous tubules showing active spermatogenesis.

The degree and stage of spermatogenesis

To count the seminiferous tubules per microscopic field, the histological slides were examined under a 80 x magnification. The counting was repeated 5 times in different areas on the same slide to obtain a representative average value. The diameter of the seminiferous tubules was measured with a graded eyepiece under 80 x magnification. Ten seminiferous tubules per microscopic field were measured while the procedure was repeated 5 times in different areas on the slide in order to obtain a representative value. Slides were placed under a 320 x magnification to measure the epithelial thickness of the seminiferous tubules. Ten tubules per microscopic field were measured, this being repeated in 5 different areas. Slides were examined under a 400 x magnification to determine the stage to which spermatogenesis had proceeded. To obtain a representative figure, 150 seminiferous tubules per slide were examined. Microphotos of the seminiferous tubules were taken at 100 and 400 x magnification.

Table 1: **The average in situ measured length (mm) and diameter (mm) of the testes in the 3 groups**

Parameter of testes	Group		
	Group C n = 9	Group M n = 8	Group MS n = 8
Length	100,1[a]	96,5[a]	69,8[b]
Diameter	115,3[a]	119[a]	93,9[b]

a,b Values within the same row with different superscripts differ significantly ($P \leq 0,05$)

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Statistical analyses

Results were analysed according to standard one way analysis of variance procedure using the P1V program of the BMDP statistical packet[4]. Differences between treatment means were tested by means of the Student's *t*-test[12].

RESULTS

Testes size and mass (Tables 1 and 2)

As shown in Table 1 the diameter and length of the testes did not differ between groups C and M.

In group MS, both measurements were smaller ($P \leq 0,05$) when compared with those of groups C and M (Table 1). There was a marked decrease in the mass of the testes (Table 2) as the copper deficiency increased.

Number, dimensions and distribution of the seminiferous tubules (Table 3 & 4, Fig 1 & 2)

In Table 3 the distribution and dimensions of the seminiferous tubules of the rams in the 3 groups are given at both 12 and 20 months of age.

According to these results (Table 3) testicular development is suppressed by a copper deficiency as measured by the development of the seminiferous tubules. This was most evident at the 12 months stage when rams in groups M and MS had already been subjected to this deficiency for a period of 4 months.

The general appearance, size and distribution of the seminiferous tubules of the 3 groups of rams at the age of 20 months are shown at 100 x magnification in Fig 1 (A, B, C).

In group C the seminiferous tubules appear active, are large in diameter and the epithelial layer is very thick. The seminiferous tubules of group M (Fig 1B) appear to be smaller when compared to those of group C and the epithelial layer appears to be thinner. The seminiferous tubules of group MS (Fig 1C) appear small and inactive with only a thin layer of epithelial cells present.

The difference in spermatogenic activity in the seminiferous tubules of the rams is summarised in Table 4 and illustrated in Fig 2 (A, B).

The normal process of spermatogenesis from the spermatogonia to the spermatozoa stage was recorded in 94% of the seminiferous tubules in group C, in 89% in group M and only in 5% of the tubules of group MS. In group MS spermatocytogenesis took place in 88% of the seminiferous tubules but was blocked after the first meiotic division. Spermatids and spermatozoa were found in only 5% of the seminiferous tubules.

The nuclei of the majority of the Sertoli cells, found in group MS were small and darkly stained, and in some cases even pycnotic, which indicates that most of the Sertoli cells in group MS were inactive. In group M and to a larger extent in group C, the Sertoli cells were very large. The fingerlike evaginations of the cytoplasm of these cells in groups C and M extended deep into the lumen of the seminiferous tubules. The nuclei of these cells were large and vesicular, an indication that these cells were active (Fig 2A). In group M and group C, spermatids and spermatozoa could be seen between and in close contact with the fingerlike evaginations of the Sertoli cells. The first stage of spermatogenesis, namely spermatocytogenesis, was probably not affected by a copper deficiency since the first stages of spermatogenesis, up to the spermatocyte stage could be found in the seminiferous tubules of all three treatment groups (Fig 1 & 2). It appears that mitosis and probably the first meiotic division also occurred in the severely copper deficient group (MS), since mi-

Table 4. **A comparison of the average (± SD) number of seminiferous tubules showing active spermatogenesis in the rams at an age of 20 months**

Group	Grade of activity			
	1	2	3	4
MS	0	88 ± 3,6	7 ± 3,5	5 ± 1,2
M	0	4 ± 4,0	7 ± 2,1	89 ± 6,1
C	0	2 ± 1	4 ± 1	94 ± 1

Grade 1 : Absence of any spermatids, spermatozoa and mitotic and meiotic divisions in seminiferous tubules

Grade 2 : Mitotic and meiotic divisions present but no spermatids or spermatozoa

Grade 3 : Mitotic and meiotic divisions present as well as spermatids but no spermatozoa

Grade 4 : Mitotic and meiotic divisions as well as spermatids and spermatozoa present in seminiferous tubules

Table 2. **The average body and testes mass of the slaughtered rams at an age of 20 months**

Treatment group	Average mass	
	Testes (g)	Body (kg)
C	424 ± 69	56 ± 5,3
M	348 ± 71	54 ± 5,3
MS	170 ± 11	41 ± 1,5

Table 3. **A comparison of seminiferous tubular development as measured by the number of seminiferous tubules per microscopic field, average tubular diameter, and thickness of tubular epithelium in the rams of the 3 treatment groups.**

Age in months	Seminiferous tubules	Group		
		C	M	MS
12	Number per microscopic field	46[a]	64[a]	109[b]
12	Diameter (μm)	120[a]	87[b]	61[c]
12	Epithelial thickness (μm)	35[a]	22[b]	14[b]
20	Number per microscopic field	35[a]	46[a]	84[b]
20	Diameter (μm)	166[a]	140[b]	77[c]
20	Epithelial thickness (μm)	49[a]	40[a]	20[b]

a,b,c Values in the same row with different superscripts differ significantly ($P \leq 0,05$)

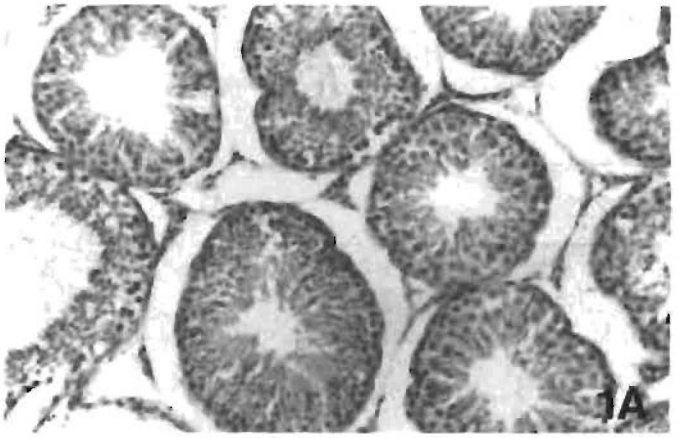

Fig. 1 A: General appearance and distribution of the seminiferious tubules in group C. HE X 100

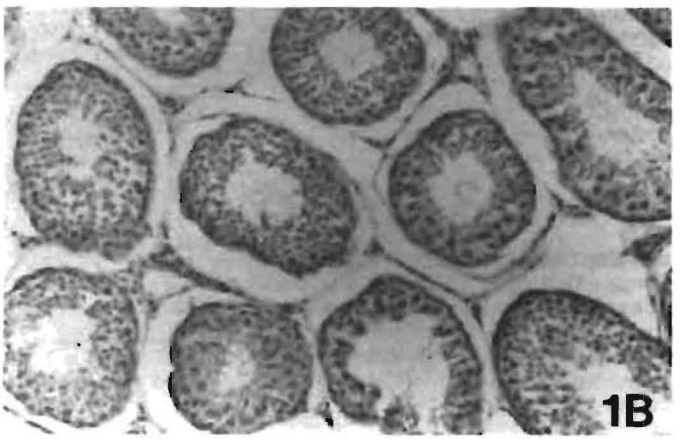

Fig. 1 B: General appearance and distribution of the seminiferous tubules in group M. HE X 100

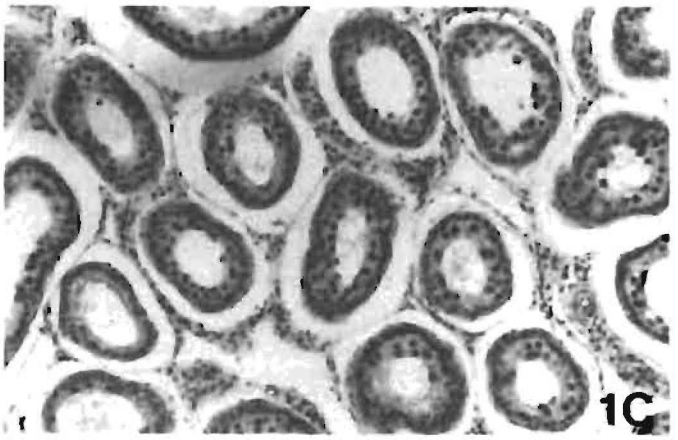

Fig. 1 C: General appearance and distribution of the seminiferous tubules in group M.S. X 100

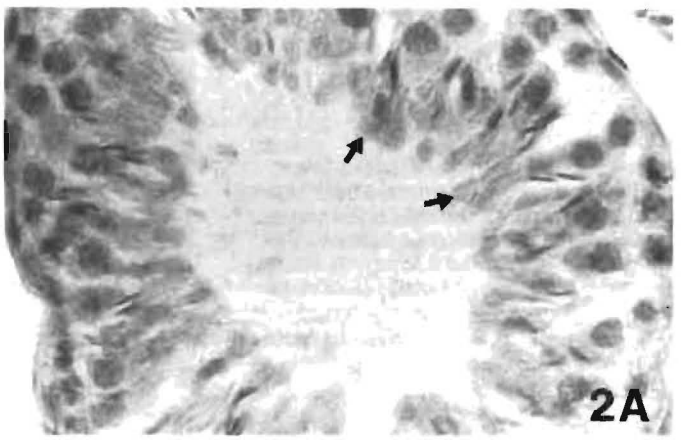

Fig. 2 A: A typical example of normal seminiferous tubules in groups C and M. with normally developed Sertoli cells. The different stages of spermato genesis are discernable. (⬅) Sertoli cells with spermatozoa embedded in its cytoplasma. HE X 400

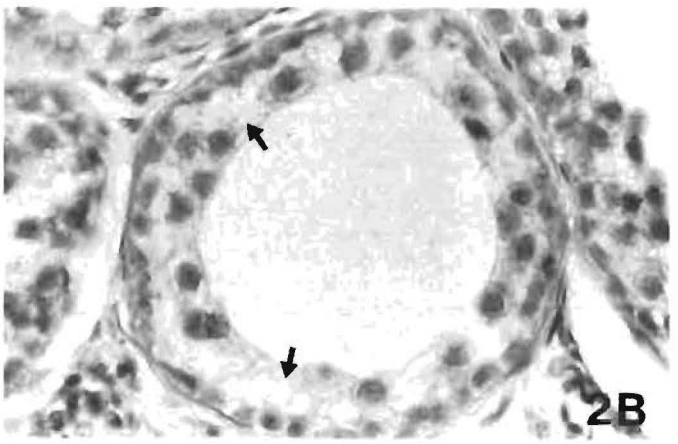

Fig. 2 B: A typical example of the seminiferous tubules in group MS with small inactive Sertoli cells. (⬅) Spermatogenesis is suspended at the spermatocyte stage. HE X 400

totic figures with dark stained, long thin thread-like chromatin were noticeable in these cells. Spermatocytes were present in almost all the seminiferous tubules of group MS (Fig 2B).

DISCUSSION

Sperm production is linked to testes size and weight[7, 14]. It can therefore be expected that the rams with the smaller testes in group M, and more so those in group MS would produce fewer sperm. This, in fact, was found to be so by Van Niekerk & Van Niekerk[17]. At the ages of 12 and 20 months the number of seminiferous tubules per microscopic field were, on average, more numerous in group MS than in groups M and C (Table 3). The average diameter of the tubules was smallest in group MS, while it was larger in group M and largest in group C. The thickness of the epithelium of the tubules followed the same pattern, namely very thin in group MS, thicker in group M and very thick in group C (Table 3, Fig 1).

All of these parameters indicate that the development and functioning of the seminiferous tubules are suppressed to some extent by a mild copper deficiency (group M), and severely suppressed by a severe copper deficiency as found in group MS. Comparing these parameters between the rams at 12 and 20 months of age, the size and activity of the seminiferous tubules in all 3 groups increased between 12 and 20 months, but to a lesser degree in the copper deficient groups M and MS. This suppression of testicular development and activity by a systemic copper deficiency cannot be regarded as a degenerative process, as described by Thomas & Moss[15], since testicular development proceeded in group MS despite a low systemic copper concentration. The abnormally low sperm production caused by the copper

deficiency in this group (MS) was found to be reversible after the copper deficiency had been corrected[17].

It is believed that the Sertoli cells play an important role in the activity of the germinal epithelium and spermatogenesis[2]. The size and activity of these cells are therefore an important indicator of activity in the germinal epithelium. Under both low (Fig 1) and high (Fig 2) magnification, the Sertoli cells of rams in group MS (Fig 1C & Fig 2B) appear inactive. The cells are small and contain a small amount of cytoplasm when compared with those found in group M and even more so when compared with those in group C (Fig 1A & B and Fig 2A).

Spermatocytogenesis is not suppressed by a copper deficiency but spermiogenesis is blocked or severely affected. During spermiogenesis the developing gametes are embedded in the Sertoli cells, which are responsible for the nutrition and nursing of these cells during the process of metamorphosis[2]. When the functioning of the Sertoli cells is impaired by a copper deficiency, spermiogenesis will be affected, resulting in low sperm production and a high percentage of abnormal spermatozoa, as found by Van Niekerk & Van Niekerk[17]. The functioning and activity of the Sertoli cells is controlled by follicle stimulating hormone (FSH)[10]. Sertoli cells also produce inhibin, which regulates the FSH secretion from the pituitary by means of a negative feedback system[1]. It is possible that the production and/or release of FSH and the resultant functioning of the Sertoli cells is suppressed by a secondary copper deficiency.

These conclusions are in agreement with the findings of several authors[3 5 13] who found that the administration of copper ions to rodents resulted in an increased production of FSH and LH. Van Niekerk & Van Niekerk[17] also reported a low testosterone concentration in a group of rams which suffered from a severe secondary copper deficiency.

It is concluded that an induced copper deficiency suppresses the development and the function of the testes in young rams. Spermatogenesis is blocked after the first meiotic division to such an extent that only a small number of spermatozoa of which a high percentage is abnormal, are produced. It is suggested that the inactivity of the Sertoli cells is responsible for the blocking of spermatogenesis. The functions of the Sertoli cells are regulated by the gonadotropic hormones. There is evidence in the literature[3 5 13] that copper might play an important role in the production and the possible release of these hormones. An impaired production of FSH would explain the inactivity of the Sertoli cells.

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BREED DIFFERENCES IN PLASMA CALCIUM, PHOSPHORUS AND MAGNESIUM CONCENTRATIONS OF MERINO, DOHNE MERINO AND S.A. MUTTON MERINO SHEEP WITH RELATION TO THE BENT-LEG SYNDROME

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ABSTRACT:

Plasma calcium, phosphorus and magnesium concentrations were determined in ewes and their single and twin lambs from birth for 120 days in Merino, Dohne Merino and South African Mutton Merino sheep. Mineral concentrations as well as the plasma calcium: phosphorus ratio were compared between breeds. Throughout the experimental period, plasma calcium and phosphorus concentrations were higher in both single and twin lambs than in the ewes of all 3 breeds. No breed differences in plasma calcium concentrations were found. Animals of the S.A. Mutton Merino breed had significantly higher ($P < 0,05$) plasma phosphorus concentrations than the other 2 breeds. The plasma Ca : P ratio in the Merino and Dohne Merino ram lambs was approximately 1,1 : 1 and in the S.A. Mutton Merino 0,9 : 1. This converse plasma Ca : P ratio found in both the S.A. Mutton Merino ewes and lambs is believed to result in an induced plasma ionised calcium deficiency which leads to improper calcification of bone. This is believed to be a contributing factor in the bent-leg syndrome. No difference was recorded in body mass between ram lambs suffering from the bent-leg syndrome and unaffected ram lambs.

Plasma magnesium concentrations were not affected by breed or age of the animals.

Key words: sheep, plasma calcium, plasma phosphorus, bent-leg syndrome

Van Niekerk F.E.; Van Niekerk C.H.; Heine E.W.P.; Coetzee J. **Breed differences in plasma calcium, phosphorus and magnesium concentrations of Merino, Dohne Merino and S.A. Mutton Merino sheep with relation to the bent-leg syndrome.** Journal of the South African Veterinary Association (1989) 60 No. 1, 36-41 (En.) Department of Human and Animal Physiology, University of Stellenbosch, 7600 Stellenbosch, Republic of South Africa.

INTRODUCTION

In South Africa a condition generally referred to as the "bent-leg syndrome" or "Bowie" occurs frequently in young lambs under a wide variety of different feeding conditions. Characteristic of this condition is the gradual bending of the front legs with the hoofs turned inwards and the knee-wrist outwards. Sometimes only one leg is affected. Present findings indicate that this condition occurs mainly in ram lambs and develops from as early as 3 months up to 1 year of age (F E van Niekerk 1987, unpublished results).

Elliot & Chrighton[4] described a similar condition in 1926 in Scotland. This condition revealed no histological signs of rickets. Malan[8] indicated that rickets could be induced by restricting the phosphate intake but was unble to provoke the same condition with a calcium-deficient diet. A long term calcium deficiency resulted in an osteodystrophic condition which is pathologically easily distinguished from rickets[8]. From the literature it is clear that the bent-leg syndrome is an osteodystrophic condition which results in improper bone calcification but is different in aetiology and must therefore be distinguished from rickets. Mineral supplementation of animals suffering from this condition should be done with care as indiscreet supplementation could aggravate the condition[6].

The "bent-leg syndrome" occurs mainly in the fast-growing sheep breeds such as the Ile de France, Dormer and S.A. Mutton Merino but also in slower growing breeds like the Merino. It is more likely to occur in stud ram lambs on a high level of feeding. In South Africa the incidence of this condition is ill-defined since most farmers are unwilling to reveal the exact figures due to fear that this condition might be of genetic origin. It is, however, believed that a great number of excellent stud animals are culled every year as a result of this condition. The objective of the present study is to investigate possible breed differences in the metabolism of certain macro minerals and the possible relationship thereof to the "bent-leg" syndrome.

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MATERIALS AND METHODS

Experimental animals and procedure

Merino (n = 74), Dohne Merino (n = 119) and South African Mutton Merino (n = 124) ewes and their lambs of the stud flocks of the Department of Sheep and Wool Science, kept on the experimental farm of the University of Stellenbosch were used. Records on the incidence of the bent-leg syndrome over the past 6 years of all 3 these breeds were available. The ewes in all 3 studs are mated in October every year. During pregnancy, all the ewes used in Phase 1 and 2 (see below) of all 3 breeds received the same feeding and treatment. Ewes grazed on wheat stubbles from 3 weeks after mating till 4 weeks prior to lambing when they were transferred to kikuyu pastures. From 6 weeks prior to lambing, all the ewes received 0,5 kg supplementary feeding daily. This was increased by 0,1 kg per week up to lambing. Supplementary diet consisted of 55% oaten hay, 35,6% barley, 4% fish meal, 3% Rumevite stud concentrate, 1% bentonite, 0,4% $NaHCO_3$, 0,2% MgO, 0,36% $Na_2S_2O_3$ and 0,41% NaCl. After the ewes had lambed, they were supplemented daily with 1,5 to 2 kg of a diet consisting of 53% lucerne, 40,3% maize, 3% fish meal, 0,35% $CaCO_3$, 2% bentonite, 0,4% $NaHCO_3$, 0,2% MgO, 0,29% $Na_2S_2O_3$ and 0,45% NaCl while they grazed on pastures consisting of kikuyu, oats, rye grass, clover and lucerne.

Phase 1: During the 1987 lambing season the experimental animals were selected one week after lambing commenced. Only ewes that had given birth within the previous 48 h were selected. The animals were selected in such a way that 7 ewes with single lambs and 7 ewes with twin lambs from each breed were included. Only the results of ewes and their lambs that remained alive throughout the trial were included. Eventually the results of 5 ewes with single lambs and 5 with twin lambs of the Merino as well as the S.A. Mutton Merino breeds were included. In the case of the Dohne Merino, 4 ewes with single lambs and 6 with twin lambs were included.

The first blood samples were taken from both these ewes and their lambs within 48 h after birth and this was repeated at 14 d intervals until weaning at approximately 120 d. All the lambs were weighed at birth, at 100 d and again at the age of 6 months. During Phase I the selected experimental animals of all 3 breeds were kept as a single flock.

Phase II: Lambs of all the ewes of all 3 breeds including the lambs used in Phase I were weaned at the approximate age of 120 d. At this stage all the lambs that did not meet the specific breed standards, except those showing sings of the "bent-leg" syndrome, were culled. After culling, all the remaining ram lambs comprising 25 Merinos, 38 Dohne Merinos and 52 S.A. Mutton Merinos were included in Phase 2. When the ram lambs reached an average age of 6 months, blood samples were taken from all the ram lambs of all 3 breeds. The ram lambs were kept as one flock under identical feeding conditions and grazed on pastures consisting of oats, rye grass, lucerne and kikuyu. They were supplement-

ed with 1 kg of a pelleted diet daily. This diet had a crude protein content of 14%, digestable organic matter of 61%, 0,71% Ca, 0,28% P and 0,26% Mg.

Blood sampling and preparation
All blood samples were taken from the vena jugularis with 18 G needles in 10 mℓ heparinised evacuated tubes (Vac-U-Test, Radem Laboratories). Two mℓ whole blood was kept at -20°C to determine the blood selenium concentration while the remaining blood was centrifuged at 3 000 r.p.m. within 3 h after collection and the plasma was removed and stored at -20°C. Extreme care was taken to avoid haemolysis of blood samples. The plasma was used for the determination of calcium, phosphorus and magnesium. The plasma copper, zinc, total protein, albumin, potassium, sodium and chloride concentrations were also determined but will be reported elsewhere.

Analytical procedures
The plasma calcium, phosphorus and magnesium concentrations were determined spectrophotometrically according to the methods used by Van Niekerk; et al[15]. Alkaline phosphatase activity was measured spectrophotometrically at 25°C by means of a standard alkaline phosphate determination kit (Clinical Sciences Diagnostics, Catalogue no C10005).

Statistics
Results were analysed according to standard one way analysis of variance procedures using the P1V program of the BMDP statistical packet[5]. Differences between treatment means were tested by t-test procedures[12]. In cases where only the average values are given, the standard deviation (± SD) of the mean is also indicated.

RESULTS
Phase I: Mean plasma calcium concentrations with standard deviations of both ewes and lambs of all 3 breeds are given in Table 1.

Table 1: **The mean plasma calcium concentrations mmol ℓ$^{-1}$ (± SD) of Merino, Dohne Merino and S.A. Mutton Merino ewes and their single or twin lambs from birth until 14 weeks of age**

Breed	Ewe or lamb and birth status	Time in weeks after parturition							
		0	2	4	6	8	10	12	14
Merino	ESL	2,79 ± 0,20	2,29 ± 0,08	2,14 ± 0,23	2,23 ± 0,22	2,39 ± 0,06	2,41 ± 0,12	2,46 ± 0,10	1,68 ± 0,53
	SL	3,17 ± 0,18	2,64 ± 0,16	2,43 ± 0,30	2,84 ± 0,08	2,65 ± 0,13	2,30 ± 0,21	3,02 ± 0,21	2,46 ± 0,24
	ETL	2,79 ± 0,18	2,27 ± 0,19	1,95 ± 0,33	2,29 ± 0,24	2,42 ± 0,16	2,45 ± 0,32	2,63 ± 0,22	2,00 ± 0,12
	TL	3,27 ± 0,33	2,70 ± 0,21	2,39 ± 0,23	2,86 ± 0,15	2,66 ± 0,04	2,54 ± 0,55	2,98 ± 0,22	2,47 ± 0,24
Dohne	ESL	2,62 ± 0,24	2,39 ± 0,09	2,09 ± 0,22	2,12 ± 0,45	2,51 ± 0,14	2,50 ± 0,20	2,67 ± 0,08	1,84 ± 0,25
Merino	SL	3,00 ± 0,05	2,68 ± 0,17	2,33 ± 0,11	2,70 ± 0,14	2,61 ± 0,12	2,46 ± 0,06	3,12 ± 0,13	2,45 ± 0,06
	ETL	2,48 ± 0,42	2,21 ± 0,09	2,11 ± 0,29	1,69 ± 0,20	2,33 ± 0,23	2,12 ± 0,30	2,55 ± 0,33	1,89 ± 0,36
	TL	3,40 ± 0,37	2,66 ± 0,23	2,44 ± 0,29	2,72 ± 0,20	2,91 ± 0,86	2,47 ± 0,29	2,91 ± 0,28	2,51 ± 0,13
S.A.	ESL	2,69 ± 0,51	2,31 ± 0,18	2,04 ± 0,10	2,20 ± 0,21	2,37 ± 0,13	1,97 ± 0,10	2,64 ± 0,18	1,82 ± 0,29
Mutton	SL	3,00 ± 0,74	2,63 ± 0,08	2,46 ± 0,17	2,62 ± 0,10	2,63 ± 0,10	2,43 ± 0,18	2,81 ± 0,23	2,48 ± 0,74
Merino	ETL	2,58 ± 0,26	2,19 ± 0,08	2,03 ± 0,19	2,18 ± 0,21	2,21 ± 0,27	2,03 ± 0,34	2,57 ± 0,29	1,71 ± 0,22
	TL	3,08 ± 0,36	2,59 ± 0,09	2,54 ± 0,30	2,84 ± 0,21	2,65 ± 0,08	2,23 ± 0,41	2,78 ± 0,20	2,35 ± 0,17

ESL — Ewes with single lambs
SL — Single lambs
ETL — Ewes with twin lambs
TL — Twin lambs

Table 2: **A comparison of the mean total plasma calcium concentration (mmol ℓ$^{-1}$) in adult Merino, Dohne Merino and S.A. Mutton Merino ewes during lactation.**

Weeks after paturition	Merino	Dohne Merino	SA Mutton Merino
0	2,79^a	2,54^a	2,63^a
2	2,28^a	2,28^a	2,25^a
4	2,05^a	2,10^a	2,04^a
6	2,26^a	1,86^b	2,19^a
8	2,41^a	2,40^a	2,29^a
10	2,43^a	2,27^a	1,99^b
12	2,55^a	2,59^a	2,60^a
14	1,84^a	1,87^a	1,76^a

a,b Values in the same row with different headings differ significantly ($P \leq 0,05$)

Throughout the preweaning period, both single and twin lambs of all 3 breeds had higher plasma calcium concentrations than the ewes. Plasma calcium levels did not differ significantly between single and twin lambs. Giving birth to a single lamb or twins had no influence on the plasma calcium concentrations of the ewe. There was statistically no significant breed differences in plasma calcium concentrations of the ewes (Table 2) or the lambs of the 3 breeds (Table 3).
Mean plasma inorganic phosphorus concentrations with standard deviations of the ewes and lambs of all 3 breeds are given in Table 4.

Plasma inorganic phosphorus concentrations (PIP) did not differ significantly between single and twin lambs. As was the case with calcium, the number of lambs born per ewe had no influence on the PIP concentrations of the ewe. However, at birth the mean PIP concentrations of the

Table 3: **A comparison of the mean total plasma calcium concentration (mmol ℓ^{-1}) in Merino, Dohne Merino and S.A. Mutton Merino lambs *before* weaning**

Age (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	3,23[a]	3,30[a]	3,06[a]
2	2,69[a]	2,67[a]	2,60[a]
4	2,41[a]	2,41[a]	2,45[a]
6	2,85[a]	2,72[b]	2,77[a,b]
8	2,65[a]	2,83[a]	2,64[a]
10	2,46[a]	2,47[a]	2,30[a]
12	3,00[a]	2,96[a]	2,79[b]
14	2,47[a]	2,49[a]	2,39[a]

a,b Values in the same row with different headings differ significantly ($P \leq 0,05$)

lambs of all 3 breeds were approximately 3 times higher than those of the ewes. As the lambs grew older, their PIP concentrations decreased while those of the ewes increased. The average PIP concentrations of the Merino lambs decreased from 3,39 to 2,45 mmol ℓ^{-1} (Table 6) while those of the Merino ewes increased from 1,24 mmol ℓ^{-1} to 1,74 mmol ℓ^{-1} (Table 5) during the same period. The average PIP concentrations of the Dohne Merino lambs decreased from 3,51 mmol ℓ^{-1} to 2,42 mmol ℓ^{-1} (Table 6) while those of the Dohne Merino ewes increased from 1,24 to 1,51 mmol ℓ^{-1} (Table 5). Noteworthy is the fact that the S.A. Mutton Merino lambs revealed the smallest decrease in PIP concentrations of the 3 breeds namely from 3,51 mmol ℓ^{-1} to 2,86 mmol ℓ^{-1} (Table 6). The PIP concentrations of the S.A. Mutton Merino ewes increased from 1,06 mmol ℓ^{-1} to 1,88 mmol ℓ^{-1} (Table 5).

Table 4: **The mean plasma inorganic phosphorus concentrations (mmol ℓ^{-1} ±SD) in Merino, Dohne Merino and S.A. Mutton Merino ewes and their single or twin lambs from birth until 14 weeks of age**

Breed	Ewe or lamb and birth status	Time in weeks after parturition							
		0	2	4	6	8	10	12	14
Merino	ESL	1,38 ± 0,35	1,41 ± 0,15	1,72 ± 0,32	1,89 ± 0,24	1,59 ± 0,30	1,74 ± 0,27	1,78 ± 0,18	1,79 ± 0,11
	SL	3,59 ± 0,46	3,34 ± 0,19	3,07 ± 0,22	3,11 ± 0,27	2,73 ± 0,23	2,87 ± 0,48	2,86 ± 0,67	2,61 ± 0,35
	ETL	1,09 ± 0,27	1,39 ± 0,31	1,63 ± 0,20	1,74 ± 0,41	1,61 ± 0,30	1,97 ± 0,34	1,53 ± 0,31	1,69 ± 0,29
	TL	3,18 ± 0,32	3,43 ± 0,30	2,70 ± 0,27	3,14 ± 0,32	2,66 ± 0,36	2,86 ± 0,40	2,59 ± 0,41	2,29 ± 0,18
Dohne	ESL	1,28 ± 0,15	1,44 ± 0,18	1,32 ± 0,15	1,56 ± 0,25	1,31 ± 0,31	1,60 ± 0,25	1,64 ± 0,16	1,41 ± 0,06
Merino	SL	3,36 ± 0,14	3,33 ± 0,19	3,16 ± 0,37	2,89 ± 0,13	2,66 ± 0,45	2,86 ± 0,28	2,48 ± 0,34	2,31 ± 0,30
	ETL	1,22 ± 0,33	1,11 ± 0,25	1,61 ± 0,31	1,93 ± 0,35	1,64 ± 0,24	1,85 ± 0,25	1,84 ± 0,32	1,59 ± 0,27
	TL	3,66 ± 0,60	3,31 ± 0,50	2,76 ± 0,35	3,23 ± 0,36	2,58 ± 0,23	2,92 ± 0,29	2,63 ± 0,34	2,53 ± 0,33
S.A.	ESL	1,14 ± 0,44	1,48 ± 0,18	1,91 ± 0,24	2,03 ± 0,39	1,66 ± 0,29	2,07 ± 0,44	2,24 ± 0,39	1,81 ± 0,25
Mutton	SL	4,08 ± 0,51	3,47 ± 0,26	3,37 ± 0,28	3,47 ± 0,21	3,00 ± 0,23	3,31 ± 0,34	3,25 ± 0,34	2,99 ± 0,33
Merino	ETL	0,99 ± 0,30	1,24 ± 0,17	1,53 ± 0,12	2,16 ± 0,45	1,69 ± 0,34	2,55 ± 0,67	2,06 ± 0,21	1,95 ± 0,30
	TL	2,94 ± 0,62	3,18 ± 0,22	2,79 ± 0,34	3,27 ± 0,31	2,86 ± 0,45	3,05 ± 0,48	3,13 ± 0,54	2,74 ± 0,35

ESL — Ewes with single lambs
SL — Single lambs
ETL — Ewes with twin lambs
TL — Twin lambs

Table 5: **A comparison of the mean plasma inorganic phosphorus concentrations (mmol ℓ^{-1}) in adult Merino, Dohne Merino and S.A. Mutton Merino ewes during lactation**

Weeks after parturition	Merino	Dohne Merino	SA Mutton Merino
0	1,24[a]	1,24[a]	1,06[a]
2	1,40[a]	1,24[a]	1,36[a]
4	1,68[a]	1,49[a]	1,72[a]
6	1,82[a]	1,78[a]	2,09[a]
8	1,60[a]	1,50[a]	1,67[a]
10	1,86[a]	1,75[a]	2,31[a]
12	1,66[a]	1,75[a]	2,15[b]
14	1,74[a]	1,51[b]	1,88[a]

a,b Values in the same row with different headings differ significantly ($P \leq 0,05$)

When the PIP concentrations of the adult ewes of the 3 breeds are compared (Table 5) it is found that the PIP values of the S.A. Mutton Merino were on average higher than those of the other 2 breeds from 4 weeks post partum and onwards.

When the PIP concentrations of the lambs are compared (Table 6) it is found that after the age of 4 weeks, the values of the S.A. Mutton Merino lambs were higher ($P < 0,05$) than those found in the other 2 breeds.

The plasma Ca : P ratio of the adult ewes of the 3 breeds during lactation is shown in Table 7.

At birth, ewes of all 3 breeds had a wide Ca : P ratio of more than 2 : 1 but as lactation proceeded, the ratio declined. In the case of the Merino breeds the calcium concentration always exceeded that of the PIP concentration whereas in the case of the S.A. Mutton Merino this ratio declined to such an extent that the calcium concentration was exceeded by that of the PIP

concentration to give a converse ratio of 0,94 : 1.

The plasma Ca : P ratio of the lambs of the 3 breeds is given in Table 8.

The plasma Ca : P ratio in lambs of the Merino and Dohne Merino breeds increased from approximately 0,95 : 1 to 1,09 : 1 at the age of 12 weeks. At the age of 12 weeks the calcium concentrations exceeded PIP concentrations. However the opposite was found in the S.A. Mutton Merino lambs where the plasma Ca : P ratio declined from 0,92 : 1 at birth to 0,84 : 1 at the age of 14 weeks.

Phase 2: During the present experimental period 15 S.A. Mutton Merino ram lambs out of a total of 81 weaned (18,5%) developed the bent-leg syndrome before the age of 6 months. Again this condition did not occur in the ewe lambs of this breed or in both the ewe and ram lambs of the Merino and Dohne Merino breeds during the present experimental period.

The converse plasma Ca : P ratio in the S.A. Mutton Merino is confirmed when the concentrations of these minerals, which were determined in the ram lambs at the age of 6 months, are taken into consideration (Table 9).

At the age of 6 months the plasma Ca : P ratio in the Merino ram lambs were 1,14 : 1, Dohne Merino ram lambs 1,13 : 1 and S.A. Mutton Merino ram lambs 0,91 : 1.

No significant differences could be found in the plasma calcium, PIP and magnesium concentrations (Table 10) between S.A. Mutton Merino ram lambs with normal and those with affected (bent-leg) legs at the age of 6 months.

As seen in Table 11, no difference in body mass was recorded between S.A. Mutton Merino ram lambs with normal and those with abnormal legs.

Rams with affected legs, had on average, a low plasma alkaline phosphatase activity when compared to that of the rams with normal legs (Table 12).

From the mean plasma magnesium (± SD) concentrations (Phase I) of the ewes and their lambs of the 3 breeds recorded from birth to weaning (Table 13), it was clear that there was virtually no difference between ewes and lambs. Birth status and breed had no influence on the plasma magnesium concentrations.

Table 6: **A comparison of the mean total plasma calcium concentration (mmol ℓ^{-1}) in Merino, Dohne Merino and S.A. Mutton Merino lambs before weaning**

Age (weeks)	Merino	Dohne Merino	Merino	SA Mutton Merino
0	3,39[a]	3,51[a]		3,51[a]
2	3,40[a]	3,32[a]		3,28[a]
4	2,82[a]	2,86[a]		2,98[a]
6	3,13[a]	3,14[a]		3,34[b]
8	2,68[a]	2,60[a]		2,90[b]
10	2,86[a]	2,91[a]		3,14[b]
12	2,68[a]	2,60[a]		3,17[a]
14	2,45[a]	2,42[a]		2,86[b]

a,b Values in the same row with different headings differ significantly ($P \leq 0,05$)

Table 7: **The plasma Ca:P ratio of adult Merino, Dohne Merino and S.A. Mutton Merino ewes during lactation**

Weeks after parturition	Merino	Dohne Merino	SA Mutton Merino
0	2,25:1	2,05:1	2,48:1
2	1,63:1	1,84:1	1,65:1
4	1,22:1	1,41:1	1,60:1
6	1,24:1	1,04:1	1,04:1
8	1,50:1	1,60:1	1,37:1
10	1,30:1	1,30:1	0,86:1
12	1,54:1	1,48:1	1,20:1
14	1,05:1	1,24:1	0,94:1

Table 8: **The plasma Ca:P ratio of the Merino, Dohne Merino and S.A. Mutton Merino lambs from birth to 14 weeks of age**

Age (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	0,95:1	0,94:1	0,86:1
2	0,79:1	0,80:1	0,79:1
4	0,85:1	0,84:1	0,82:1
6	0,91:1	0,87:1	0,82:1
8	0,99:1	1,09:1	0,91:1
10	0,86:1	0,84:1	0,73:1
12	1,04:1	1,13:1	0,88:1
14	1,03:1	0,98:1	0,81:1

DISCUSSION

According to records kept over the past 6 years, as many as 20% of the ram lambs born from the S.A. Mutton Merino ewes of the University's stud, developed the bent-leg syndrome. However none of the ewe lambs developed this condition. In contrast, no ram or ewe lambs of the Merino stud kept on the same farm and under the same feeding conditions, developed the bent-leg syndrome. Although no Dohne Merino ewe lambs developed this syndrome, the incidence thereof in the ram lambs was very low (0 to 5%) during this time. During the present experiment 18,5% of the S.A. Mutton Merino ram lambs developed the bent-leg syndrome while none of the ram lambs of the other 2 breeds or the ewe lambs of any of the 3 breeds were affected.

The average total plasma calcium concentrations of the ewes of all three breeds varied within the normal limits of 2,25 and 2,75 mmol ℓ^{-1} during lactation[14]. The plasma calcium concentrations of the lambs of all 3 breeds were high at birth[13]. This is in agreement with the findings of Delivoria-Papadopoulos et al.[3] who found that the foetus maintained a higher plasma calcium concentration than the ewe. These high calcium concentrations of lambs can be maintained for up to 1 month, after which they decrease to the concentrations found in adults[7]. No breed differences in plasma calcium concentrations were observed in ewes or in lambs of these 3 breeds.

Normal PIP concentrations[14] for adult sheep vary between 1,29 and 1,94 mmol ℓ^{-1} and those of young animals between 1,94 and 2,58 mmol ℓ^{-1}. According to these values, the ewes of the 3 breeds had low PIP levels just after birth in contrast with those of the lambs which were abnormally high (Table 4). It was found that

Table 9: **The mean serum calcium, phosphorus and magnesium concentrations (mmol ℓ^{-1} in Merino, Dohne Merino and S.A. Mutton Merino ram lambs with normal legs at 6 months of age**

Mineral	Merino n = 25	Dohne Merino n = 39	S.A. Mutton Merino n = 37
Calcium	2,99[a]	2,93[a]	2,71[b]
Phosphorus	2,61[a]	2,59[a]	2,95[b]
Magnesium	0,78[a]	0,73[b]	0,74[b]

a,b Values in the same row with different headings differ significantly ($P < 0,05$)

Table 10: **The mean serum calcium, phosphorus and magnesium concentrations (mmol ℓ^{-1} in S.A. Mutton Merino rams with normal and abnormal legs at 6 months of age**

Mineral	Normal legs n = 37	Bent-legs n = 15
Calcium	2,71[a]	2,72[a]
Phosphorus	2,95[a]	2,97[a]
Magnesium	0,74[a]	0,72[a]

a,b Values in the same row with different headings differ significantly ($P < 0,05$)

Table 11 **The mean body mass in kg (±SD) at 100 and 180 d of age of the Merino, Dohne Merino and S.A. Mutton Merino ram lambs with normal and abnormal legs**

Age (days)	Merino* n = 25	Dohne Merino* n = 38	S.A. Mutton Merino Normal legs n = 37	S.A. Mutton Merino Bent-legs n = 15
100	31,48±3,44	33,22±3,86	34,27±4,44	34,29±4,28
180	48,80±5,08	51,10±4,59	55,53±4,35	55,43±4,08

* No rams developed the bent-leg syndrome in these breeds

Table 12: **The mean plasma alkaline phosphatase activity in the S.A. Mutton Merino rams with normal and abnormal legs.**

	Normal legs	Bent-legs
Number of rams	20	10
Alkaline phosphatase IU ℓ^{-1}	250 ± 58	207± 73
Range	169 — 355	93 — 325

the S.A. Mutton Merino ewes and lambs of this stud have higher plasma PIP concentrations than the Merino and Dohne Merino breeds during lactation. The 3 breeds were kept under identical feeding conditions on the same farm.

Swenson[13] indicated that a normal serum Ca : P ration for sheep should be between 1,15 — 2,25 : 1. As seen in Tables 6 & 7 this was the case in the Merino and Dohne Merino breeds. However the S.A. Mutton Merino had a converse plasma Ca : P ratio. Despite the high initial PIP concentrations, the plasma Ca : P ratio of the Merino and Dohne Merino lambs attained normal levels after they had reached the age of 10 weeks while that of the S.A. Mutton Merino lambs declined even further. When the lambs were 6 months old, this converse plasma Ca: P ratio (0,91 : 1) in the S.A. Mutton Merino still existed.

The higher PIP concentrations found in the S.A. Mutton Merino ewes and lambs, when compared to those of the other 2 breeds, are suspected as being one of the contributing factors which cause the bent-leg syndrome in the S.A. Mutton Merino ram lambs. A prerequisite for normal bone calcification is the presence in the plasma of sufficient calcium and phosphorus in the right proportions as calcification of the bone matrix results from the deposition of these inorganic salts derived from the plasma[1]. Plasma calcium consists of two fractions of which one is bound mainly to albumin and the other referred to as the free or ionised fraction. Ionised plasma calcium is the physiological active part of the total plasma calcium concentration[2]. Belonje[1] indicated that the total plasma calcium concentration is an indication of changes in the ionised calcium concentration under abnormal conditions. Almost all the plasma phosphorus is present as orthophosphate ions[16]. Approximately 80% of the orthophosphate is in the form of the divalent anion HPO_4^{2-} and about 18% as the monovalent anion $H_2PO_4^{-}$[13]. In plasma, part of the Ca^{2+} binds with HPO_4^{2-} to form $CaHPO_4$. A high plasma phosphorus concentration results in the binding of a high percentage of the plasma calcium present[9]. This leads to a secondary, induced ionised calcium deficiency if a high plasma phosphate concentration is present. Although the total plasma calcium concentration is adequate, the biological active ionised plasma calcium is deficient under these circumstances. This results in either the extraction of Ca from the bones or the limitation of the calcification process of bones when this converse Ca : P ratio exists as found in the S.A. Mutton Merino stud in this experiment.

Alkaline phosphatase is one of the enzymes involved in the calcification of bones[11]. According to the average alkaline phosphatase activity and the minimum values obtained (Table 12), ram lambs that suffered from the bent-leg syndrome had indeed lower concentrations than animals with normal legs. Serum alkaline phosphatase activity is also affected by the R-O-i blood group system of sheep[10]. Alkaline phosphatase activity in sheep with blood group O, is approximately 75% higher than that in sheep with blood group R. Although results concerning blood group i are limited, there are indications that this group resembles group R as far as alkaline phosphatase activity is concerned. However no conclusion could be drawn regarding the effect of alkaline phosphatase activity in

Table 13: **The mean plasma magnesium concentrations mmol ℓ^{-1} (± SD) of Merino, Dohne Merino and S.A. Mutton Merino ewes and their single or twin lambs from birth until 14 weeks of age**

Breed	Ewe or lamb and birth status	Time in weeks after parturition							
		0	2	4	6	8	10	12	14
Merino	ESL	0,80 ± 0,10	0,92 ± 0,07	0,89 ± 0,15	0,92 ± 0,07	0,97 ± 0,12	0,96 ± 0,11	1,04 ± 0,08	1,02 ± 0,09
	SL	0,79 ± 0,10	0,86 ± 0,11	0,91 ± 0,09	0,86 ± 0,10	0,84 ± 0,16	0,94 ± 0,13	0,91 ± 0,09	0,94 ± 0,08
	ETL	0,84 ± 0,07	0,92 ± 0,06	0,88 ± 0,23	0,87 ± 0,06	0,95 ± 0,06	0,89 ± 0,06	1,02 ± 0,04	0,88 ± 0,09
	TL	0,68 ± 0,05	0,78 ± 0,09	0,77 ± 0,05	0,74 ± 0,05	0,78 ± 0,05	0,84 ± 0,05	0,84 ± 0,09	0,98 ± 0,21
Dohne	ESL	0,80 ± 0,09	0,98 ± 0,02	0,86 ± 0,04	0,90 ± 0,10	0,91 ± 0,09	0,86 ± 0,11	0,97 ± 0,09	0,93 ± 0,06
Merino	SL	0,69 ± 0,02	0,78 ± 0,08	0,79 ± 0,06	0,73 ± 0,05	0,81 ± 0,07	0,82 ± 0,05	0,85 ± 0,12	1,04 ± 0,11
	ETL	0,78 ± 0,12	0,97 ± 0,11	0,84 ± 0,04	0,90 ± 0,10	0,96 ± 0,09	0,99 ± 0,11	1,11 ± 0,15	0,82 ± 0,17
	TL	0,73 ± 0,06	0,71 ± 0,07	0,75 ± 0,06	0,73 ± 0,06	0,79 ± 0,05	0,80 ± 0,09	0,79 ± 0,07	0,89 ± 0,15
S.A.	ESL	0,72 ± 0,09	1,01 ± 0,06	0,87 ± 0,06	0,94 ± 0,09	1,01 ± 0,05	0,93 ± 0,06	1,05 ± 0,10	0,98 ± 0,07
Mutton	SL	0,71 ± 0,17	0,75 ± 0,05	0,82 ± 0,06	0,71 ± 0,11	0,82 ± 0,03	0,81 ± 0,03	0,81 ± 0,07	0,98 ± 0,30
Merino	ETL	0,76 ± 0,07	1,02 ± 0,13	0,90 ± 0,60	0,98 ± 0,06	1,06 ± 0,07	0,98 ± 0,10	1,01 ± 0,10	0,96 ± 0,12
	TL	0,69 ± 0,09	0,82 ± 0,08	0,81 ± 0,10	0,79 ± 0,06	0,85 ± 0,06	0,85 ± 0,09	0,90 ± 0,10	0,99 ± 0,19

ESL — Ewes with single lambs
SL — Single lambs
ETL — Ewes with twin lambs
TL — Twin lambs

respect of the bent-leg syndrome in the present study.

There were no significant differences in plasma magnesium concentrations between breeds (Table 13) and average values varied[12] between the normal limits of 0,74 and 1,31 mmol ℓ^{-1}. Plasma copper and zinc and whole blood selenium concentrations did not seem to be contributing factors to the bent-leg syndrome (F E van Niekerk 1988 Unpublished results).

A statement often made in the past is that extraordinary mass gain is the main cause of the bent-leg syndrome. It was however found in this study that in fact rams with affected legs, revealed no increased body mass as compared to unaffected rams (Table 10) at any stage.

As only ram lambs are affected by the bent-leg syndrome, the possibility exists that the factors causing this problem might be sex-linked. It seems that the high PIP concentration results in an induced ionised calcium deficiency which eventually leads to improper bone mineralisation. The possibility remains that there might be some genetic factors within a breed which make these animals more susceptible to this condition. The fact that the calcium and phosphorus ratio was found to be altered in the S.A. Mutton Merino in this specific stud, does not mean that these "genetic factors" prevail in this breed only. Further investigations on several S.A. Mutton Merino studs will have to be done before this converse plasma Ca : P ratio found in the University's stud can be regarded as a breed characteristic.

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VALIDATION OF THE TRITIATED WATER DILUTION TECHNIQUE USED TO ESTIMATE FRACTIONAL TURNOVER RATES OF BODY WATER IN SHEEP

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ABSTRACT

Daily fractional water turnover rate (i.e. that proportion of total body water which is exchanged daily) was determined in S.A. Mutton Merino sheep (n = 6) under thermoneutral conditions by direct measurement of total water intake and by the tritiated water dilution technique. No significant difference between direct and indirect methods was observed in any of the animals. Tritiated water space estimated from a plasma sample taken at 6 h after tritium administration in general overestimated this compartment when compared with values derived from the zero time intercept of the linear regression analysis obtained for each animal.

Key words: Total body water, water turnover rate, tritiated water, sheep

Meintjies R.S.: Minnaar P.P. Validation of the tritiated water dilution technique used to estimate fractional turnover rates of body water in sheep. *Journal of the South African Veterinary Association* **(1989) 60 No. 1, 42-48 (En.) Department of Physiology, Faculty of Veterinary Science, University of Pretoria, P/Bag X04, 0110 Onderstepoort, Republic of South Africa.**

INTRODUCTION

Of the various techniques (urea, sulphonamides, antipyrine, isotopic water) that have been developed to determine total body water and the kinetics of this pool, those using tritiated and deuterated water as markers have emerged as the method of choice[7]. Substances such as antipyrine are rapidly metabolised and require serial sampling during total body water estimation studies[18]. Urea space fairly accurately reflects total body water and the use of urea as a marker has definite advantages over tritiated water in that it is cheaper than the latter and furthermore it is non-radioactive, so that no special facilities are required for its application and it can be used in animals destined for human consumption[12]. Markers which are rapidly excreted (such as urea) and rapidly metabolisable substances (antipyrine) would be of little use in following water turnover rates over any appreciably period. Water labelled with a radio-active isotope, unlike the non-water tracers, behaves almost identically to body water and is neither rapidly metabolised nor rapidly eliminated from the body water pool in which it becomes uniformly dispersed[7].

The distribution of tritiated water, following intravenous administration, is a function of its transport within the circulation and its diffusion from the circulation through the interstitium to the intracellular space in monogastric species. It has been shown in dogs that the distribution of isotope in low blood flow, large volume compartments eg. hindlimb musculature, is relatively slow and requires a period of 1 to 2 h after isotope administration for complete equilibration to be achieved[3] (Equilibration in this sense is defined as the uniform distribution of isotope throughout the total body water pool).

In the ruminant, however, the equilibration time is longer, as the isotope must in addition distribute throughout the ruminal water compartment, which may account for up to 30% or more of the total body water[17]. The equilibration period has been estimated to be between 5 and 8 h by various researchers[9 16]. In general however, an equilibration period of 6 to 6,5 h following intravenous injection of isotope is accepted in ruminants[11 15].

The dilution of tritiated water throughout the total body water pool (when the latter is considered as a one compartmental steady state system) follows an exponential curve with time[7]. The decline in specific activity of the marker over a period of time is ascribed to its loss from the animal via excretion and evaporation and the concurrent input of unlabelled water by means of oxidative metabolism, eating and drinking[13].

The use of tritiated water in the estimation of total body water has been validated by several researchers in several animal species. Such studies have inevitably involved the dessication of the carcases of experimental subjects[11 14 15 17]. Validation of the tritiated water technique in predicting water turnover rates has however received limited attention[2].

The validation, in sheep, of the fractional turnover rate of body water (i.e. that proportion of total body water which is exchanged per unit time) was the primary objective of this trial.

MATERIALS AND METHODS

Animals

S.A. Mutton Merino wethers (approximately 2 years old) (n = 6), with body mass ranging from 42 to 62 kg, were individually confined in metabolic crates, in a room where ambient air temperature varied from a minimum of 9°C to a maximum of 16°C during the experimental period. The animals were translocated from an outside pen to the crates about 2 weeks prior to the commencement of the trial, at which time a diet of milled lucerne hay was offered ad libitum and water was freely available for the duration of the adaptation period. The feed was milled, mixed and then bagged for the entire duration of the trial.

Experimental design and procedure

For the purpose of this experiment the animals were assumed to be in a stable condition regarding their total body water pool, i.e. water in = water out. Daily total water intake (feed water, drinking water and metabolic water) was therefore taken as indicative of daily water flux by direct measurement.

During the experimental period, which lasted 14 d, water and feed were available ad libitum and intakes of both were recorded daily. Water intake was corrected for evaporative loss from the drinking troughs.

The faeces produced by each animal was collected, weighed and recorded daily.

The animals were weighed every second day.

On Day 1 of the trial, approximately 15-16 ml of tritiated water (activity $3,7 \times 10^4$ $Bq.ml^{-1}$) was injected into the left jugular vein of each sheep. In each case, a 20 ml syringe containing the approximate dose was weighed with needle, and following administration of the isotope without back flushing with blood, the weight of the empty syringe and needle was determined. The administered dose was calculated by determining the difference.

Five ml blood samples, were drawn into evacuated tubes containing EDTA (Venoject, Terumo Corporation, Tokyo, Japan) from the right jugular vein of each sheep at 6 h post injection (Day 1) and then daily between 08h00 and 09h00 for the duration of the experiment. The plasma was separated by centrifugation, collected and stored at 4°C awaiting further analysis.

Analyses

A "grab" sample of the contents of each bag of feed utilised during the trial was obtained. Samples from two successive bags were pooled for the analysis of moisture[1], crude protein[6] and non-protein organic matter content[6].

A 10% sample of the daily faecal output was taken and pooled on a 4-day basis. Contents of moisture[1], crude protein[6] and non-protein organic matter[6] were determined on these pooled samples.

The radio-activity (in counts per minute (cpm)) of the injected tritiated water was established by diluting the stock solution one 100-fold and by counting 6 replicate samples of 0,2 ml each in a liquid scintillation spectrometer (A Packard Liquid Scintillation Spectrometer Model 3385, Packard, Illinois, USA).

Water was obtained from the plasma samples by vacuum sublimation and two aliquots of (0,2 ml each) were mixed with scintillation fluid ("Instagel" — Packard, Il-

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Illinois USA) and the radio-activity measured in cpm. The results were expressed as the mean of 2 values corrected for background radio-activity.

Calculations

Digestible crude protein and digestible non-protein organic matter intakes were calculated for each sheep during the entire experimental period from the crude protein and non-protein organic matter contents of the feed and faeces.

Metabolic water production for each animal for the entire trial period was calculated on the basis that 396 and 556 mg of water is produced from 1 g of protein and 1 g of non-protein organic matter respectively[5].

Once the tritiated water had been fully distributed throughout the total body water pool (i.e. equilibration had been achieved — at 6 h following the isotope administration), the tritiated water space was calculated according to the following formula[7]:

$$\text{TOH space (ml)} = \frac{SdVd}{Se} - Vd$$

where TOH = tritiated water

Sd = specific concentration of tritiated water administered (Bequerel or counts per min per ml)

Vd = volume of TOH administered (ml)

Se = the specific concentration of TOH at equilibrium (Bequerel or counts per minute per ml)

Since the investigation was carried out in relatively large animals, i.e. where Vd is negligibly small in comparison to total body water, the equation may be simplified to:

$$\text{TOH space} = \frac{SdVd}{Se} \quad \text{..... Equation 1}$$

The decline in tritium concentration with time may be described by the following equation[7]:

$$\ell nSt = \ell nSo - Kt \quad \text{..... Equation 2}$$

where ℓnSt = natural logarithm of the specific concentration of tritium at time t.

ℓnSo = natural logarithm of the specific concentration of tritium at time zero.

t = time (hours or days)

K = fractional turnover rate of water i.e. that fraction of total body water exchanged hourly/daily

Using a least squares regression analysis of the specific concentrations of tritium against time, the value for K and ℓnSo may be obtained. The latter may then be used to estimate total body water by substituting So for Se in Equation 1[7].

Table 1: Moisture, crude protein and non-protein organic matter contents of feed (mean and range)

Moisture (%)	11,3 (9,1 — 14,4)
Crude protein (% of dry matter)	18,56 (17,06 — 20,18)
Non-protein organic matter (% of dry matter)	73,4 (71,8 — 74,9)

Table 2: Moisture, crude protein and non-protein organic matter contents of faeces (mean and range)

Sheep no	Moisture (%)	Crude protein (% of dry matter)	Non-protein organic matter (% of dry matter)
A	74,4(67,9-83,4)	16,1(13,8-18,6)	68,8(66,3-71,1)
B	74,8(64,1-86,4)	16,3(15,7-17,2)	69,6(68,7-70,2)
C	73,4(63,1-79,7)	17,4(16,5-19,1)	69,7(68,0-70,6)
D	76,4(72,5-85,7)	16,3(15,7-16,6)	69,0(68,7-69,6)
E	64,9(60,0-77,6)	14,3(12,9-14,6)	72,8(72,5-75,2)
F	69,5(64,6-78,9)	13,0(11,3-14,8)	74,4(73,6-76,1)

Table 3: Mean daily water gain and the standard error of the mean (in brackets) expressed as drinking water, feed water, metabolic water and total water

Sheep	Drinking water (ℓ)	Feed water (ℓ)	Metabolic water (ℓ)	Total water (ℓ)
A	6,990(±0,979)	0,241(±0,027)	0,592	7,823(±0,994)
B	8,618(±0,674)	0,258(±0,029)	0,623	9,499(±0,685)
C	5,800(±0,542)	0,230(±0,016)	0,567	6,597(±0,543)
D	7,653(±0,623)	0,253(±0,021)	0,625	8,531(±0,628)
E	5,033(±0,846)	0,189(±0,031)	0,442	5,663(±0,872)
F	5,760(±1,00)	0,256(±0,032)	0,651	6,667(±1,030)

Table 4: Tritiated water space (TOH space) expressed as a percentage of body mass

Sheep	TOH space (zero time)	TOH space (6 h)
A	67,8%	70,8%
B	69,2%	77,2%
C	70,2%	78,2%
D	66,6%	72,4%
E	70,7%	76,5%
F	67,1%	67,1%

Table 5: Daily fractional turnover rates (FTR) and mean specific water intakes determined directly and by tritiated water dilution* (mean and standard deviation)

	Daily FTR day^{-1}		Mean daily water intake ℓ kg^{-1}	
Sheep	Tritium dilution method	Direct method	Tritium dilution method	Direct method
A	0,22±(0,0036)	0,22±(0,0279)	0,150±(0,0024)	0,151±(0,0189)
B	0,25±(0,0026)	0,26±(0,0185)	0,173±(0,0018)	0,181±(0,0128)
C	0,22±(0,0033)	0,22±(0,0183)	0,155±(0,0023)	0,157±(0,0128)
D	0,22±(0,0029)	0,23±(0,0169)	0,145±(0,0019)	0,154±(0,0110)
E	0,16±(0,0028)	0,16±(0,0245)	0,116±(0,0020)	0,116±(0,0173)
F	0,17±(0,0036)	0,16±(0,0239)	0,112±(0,0024)	0,107±(0,0161)

*based on tritiated water space at zero time.

RESULTS

Analysis of feed and faeces

Analysis performed on 10 bags of feed (5 samples) used for the duration of the experiment showed little variation. The moisture, crude protein and non-protein organic matter contents appear in Table 1.

The mean values for the 4-day pooled faecal samples from each sheep for the duration of the experimental period, for moisture, crude protein and non-protein organic matter are recorded in Table 2.

Mean total daily water intakes for each sheep are recorded in Table 3 together with the individual contributions of mean daily drinking, feed and metabolic water produced. The mean daily feed water was calculated as the product of the mean moisture content of feed (11,3%) and the mean daily feed intake. Using the mean crude protein and non-protein organic matter contents of the feed and faeces respectively as well as the mean daily feed intake and faeces produced by each sheep, mean daily intakes of digestible crude protein and non-protein organic matter could be derived. From these values mean daily metabolic water production was calculated[5].

Water turnover rate and mean daily specific water intake

The exponential decline of tritium concentration in plasma samples with time as typified by that in Sheep B, is shown in Fig. 1.

i) FTR of body water per day
$y = 0{,}0303\ (\pm 0{,}012) + 0{,}839\ (\pm 0{,}0561)\ x$.
$(r = 0{,}991\ n = 6)$

where y = FTR of body water estimated by tritium dilution
X = actual FTR of body water

ii) Mean daily specific water intake
$y = 0{,}02\ (\pm 0{,}0033) + 0{,}844\ (\pm 0{,}054)\ X$
$r = 0{,}984\ (n = 6)$

where y = mean specific water intake estimated by tritium dilution ($\ell\ kg^{-1}d^{-1}$)
and X = actual mean specific water intake ($\ell\ kg^{-1}d^{-1}$)

DISCUSSION

The use of tritiated water in the estimation of total body water and in body water kinetic studies requires assumption of the following: (1) that body water space be considered as a single compartment throughout which the tracer distributes uniformly[7] (2) that the animal is in a stable condition with regard to its body water turnover i.e. total body water remains constant during the experimental period. In water kinetic studies in the equation (Equation 2) which describes the dilution of tritiated water with time, viz $\ell nSt = \ell nSo\text{-}kt$, the rate constant ($k$) may be equated to the fractional turnover rate of body water only where total body water does not change during the experimental period. If the total body water pool changes during this period k describes isotope turnover only and not water flux[13]; (3) the specific concentration of isotope in water lost from the body is equal to that in the total body water pool[13]; (4) tracer molecules or atoms do not exchange to any significant extent with non-aqueous molecules in the body[7]. Tritium ions do exchange with labile hydrogen ions of non-aqueous molecules, albeit to a small extent.[10] Culebras & Moore [4] calculated that the total non-aqueous exchangeable hydrogen in protein, carbohydrates and fat in man may amount to 5,22% of the total quantity associated with substances soluble in body water. Herein, therefore, lies a potential error in using tritiated water as a marker, in that in the estimation of total body water, the tritium space will overestimate the total body water space[18].

Tritiated water space determined from a single blood sample after equilibration has been recorded to be 2%[18], 3%[17] and 5,7%[16] higher than actual total body water when expressed as a percentage of total body mass. This discrepancy was ascribed not only to the exchange of tritium atoms with labile hydrogen atoms in non-aqueous biochemical compounds, but also to the loss of marker from the body during the equilibration period via urine, faeces and evaporation[18]. In the current trial, the TOH space, when expressed as a percentage of total body mass, at 6 h was in general higher than that obtained as an extrapolated value back to zero time.

The values obtained for tritiated water space obtained by extrapolation to zero time ranged between 60,6 and 70,7% of body mass for the 6 experimental subjects. The body water pool of ruminants seldom exceeds 75% of body mass but may vary considerably because of the high degree of negative correlation between total body water and total body fat[16]. Thus values of tritiated water space expressed as a percentage of body mass in sheep have been found to range from 48,4 to 77,8%[18] and 34,6 to 72,7%[14].

The values for fractional turnover rates of body water determined by the tritium dilution method and by measurement of total daily water intake, agreed very well with each other as is revealed by the high degree of correlation between FTR values obtained by the two methods (Table 5).

This confirms the findings of King et al[9], who found a ratio of water input to TOH turnover of 0,88 in several species of wild and domesticated ruminants. The findings also agree with those of Cameron et al[2], who established the relationship between water flux rate determined by isotopic water dilution (y) and water input per day directly measured (x) to be described by the equation:

$$y = 1{,}00\ x - 0{,}01$$

(Water flux rate is the product of total body water volume and the fractional rate constant (k)).

Fractional turnover rates of body water obtained in this experiment viz 0,16 — 0,26 per day corresponded to turnover half-times of isotope from 2,8 — 4,2 d (i.e. every 2,8 to 4,2 d half the body water pool was exchanged.

Till & Downes[18] established turnover biological t 1/2 of isotope of 3,5 — 16,3 d in sheep kept indoors in crates.

Mean specific daily water intakes varied between 107 and 181 ml $kg^{-1}\ d^{-1}$ when directly determined, and between 112 and 173 ml $kg^{-1}\ d^{-1}$ as estimated from tritiated water dilution. The correlation between the former and the latter values was high (r = 0,984). Comparable values obtained by King[8], also using tritiated water as a mar-

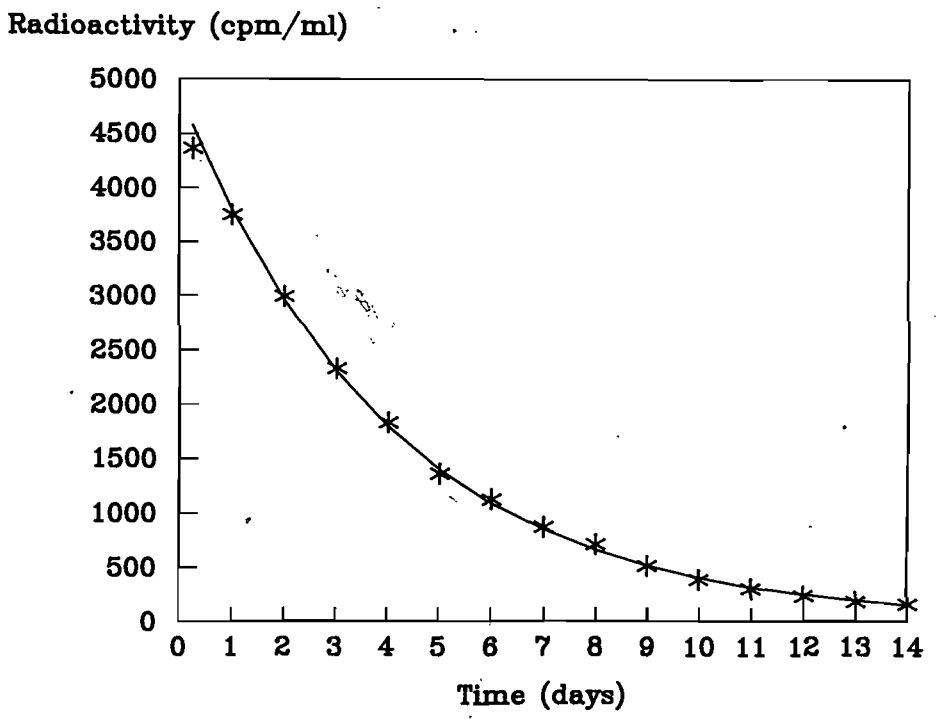

Fig. 1: The exponential decline of tritium concentration in time in plasma samples of Sheep B

——— Regression line
• Sheep B, actual data

Fractional turnover rates of body water estimated by tritium dilution varied from 0,164 to 0,25 of total body water per day (Table 5). Actual turnover rate based on the quotient of the measured mean total daily water intake and the total body water pool, taken as the extrapolated value to zero time, varied between 0,16 and 0,26 of total body water per day.

The mean specific daily water intake (i.e. mean total daily water intake per kg body mass) as derived from the tritium dilution method and by direct calculation, are recorded in Table 5. The former value is equal to FTR (per day) x TOH space (ml) at zero time per kg body mass, while the latter value is the mean measured daily water intake per kg body mass (ml $kg^{-1}\ d^{-1}$).

Comparision of actual values with those obtained by the tritium dilution method yielded the following regression equations with respect to:

ker, in a number of species of wild and domestic animals are as follows:

goat	76 — 196 ml $kg^{-1}d^{-1}$
buffalo	108 — 203 ml $kg^{-1}d^{-1}$
eland	66 — 177 ml $kg^{-1}d^{-1}$
cow	63 — 178 ml $kg^{-1}d^{-1}$
sheep	62 — 167 ml $kg^{-1}d^{-1}$
camel	38 — 76 ml $kg^{-1}d^{-1}$
oryx	30 — 124 ml $kg^{-1}d^{-1}$

The animals in King's trial were grazed on the veld during the day and corralled at night; mean ambient temperatures ranged between 24,0 and 30,4°C[8].

Water flux, even within a species, will vary considrably according to environment, activity, production, etc. Taking into account the mild ambient temperatures which prevailed during this trial, the fractional turnover rates of body water and the mean specific daily water intakes are considered to be high.

In this trial, the dilution of tritiated water in total body water with time was found to provide an easy and highly accurate means of establishing water turnover rates in sheep which are in a stable condition with respect to their total body water pool

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SUSTAINED SUPRAVENTRICULAR TACHYCARDIA IN A HORSE

A J GUTHRIE*, E NICHAS**, F VDB VILJOEN***, A M HARTMANN**** and VALERIE M KILLEEN*

ABSTRACT

A case of sustained supraventricular tachycardia of unknown aetiology in a two-year-old Thoroughbred filly is reported. The cardiac dysrhythm was successfully treated by the oral administration of quinidine sulphate. Conversion of the dysrhythm to sinus rhythm occurred approximately 80 min after the initial dose of 5 g of quinidine sulphate. The horse returned to training approximately 2 months after treatment and has since successfully returned to racing.

Key words: supraventricular arrhythmia, junctional arrhythmia, tachycardia, horse, electrocardiography, therapy

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INTRODUCTION

Junctional and ventricular arrhythmias are considered abnormal in horses and are indicative of cardiac disease or a systemic or drug-induced abnormality of cardiac rhythm[1,3,4,5]. Arrhythmias can occur secondary to fever, sepsis, electrolyte imbalances, acidosis, hypoxaemia or severe pulmonary disease, anaesthesia or the administration of arrhythmogenic drugs[2,3]. If secondary causes of arrhythmias can be ruled out, then a diagnosis of primary heart disease is appropriate[1].

Sustained junctional or ventricular tachycardias may be life-threatening arrhythmias, especially when the ventricular rate is in excess of 90 beats per min. These conditions may lead to the development of hypotension, myocardial ischaemia, syncope, seizures and shock. Due to the electrical instability associated with these conditions they may proceed to ventricular fibrillation and acute death[1].

Lignocaine hydrochloride is a local anaesthetic agent that is used as an antiarrhythmic agent in man, dogs and horses. It produces its effect by decreasing cardiac impulse conduction in diseased or depressed cardiac tissue, increasing abnormal cardiac automaticity and eliminating large differences in myocardial refractoriness. Intravenous administration of lignocaine is especially effective in the therapy of ventricular arrhythmias in horses and is particularly useful for short-term control of such arrhythmias. Lignocaine is not particularly effective in the treatment of supraventricular arrhythmias. The dosage of lignocaine required to treat ventricular arrhythmias is variable in horses and should be individualised. An intravenous dose of 0,5 to 4,0 mg kg^{-1} is recommended[6,7]. Quinidine, a drug often used for the treatment of arrhythmias in horses, prolongs cardiac refractoriness by causing a delay in the rate of conduction of the cardiac impulse and by decreasing cardiac excitability. Quinidine sulphate is dosed as a suspension in water via a nasogastric tube at a dosage rate of 20 mg kg^{-1} at 2 h intervals until a total dose of 60 g is administered or signs of toxicity are observed. Changes in behaviour, diarrhoea and anorexia are the most common side-effects observed with oral administration of quinidine[7].

CASE REPORT

A 2 year-old Thoroughbred filly in training for racing was presented for examination with a history of lethargy, a fever reaction as well as evidence of a positive jugular pulse. On clinical examination the horse appeared lethargic and anorexia was noted. A rapid positive bilateral jugular pulse was visible. The mucous membranes were congested and generalised congestion and distention of the subcutaneous vasculature, a rectal temperature of 40,2°C, tachypnoea and tachycardia (approximately 120 beats per min) were present. Auscultation of both lung fields and the cardiac field did not reveal any abnormal sounds. Although abdominal sounds were markedly reduced, the findings on rectal examination were unremarkable.

A complete blood count did not reveal any abnormalities and blood electrolyte concentrations were within normal limits. The lactate dehydrogenase, aspartate transaminase and creatine kinase activities were 1 525, 2 105 and 2 660 U/ℓ respectively (all markedly above normal values). The alpha-hydroxybutyrate dehydrogenase activity was also elevated (504 U/ℓ suggesting that the enzymes were probably of myocardial origin. Blood gas analysis of the arterial blood revealed the presence of a mild respiratory alkalosis without any evidence of hypoxaemia.

An electrocardiogram (EKG), recorded using the 3 standard limb leads, revealed the presence of QRS and T complexes of normal shape and duration that were not preceded by normally conducted P

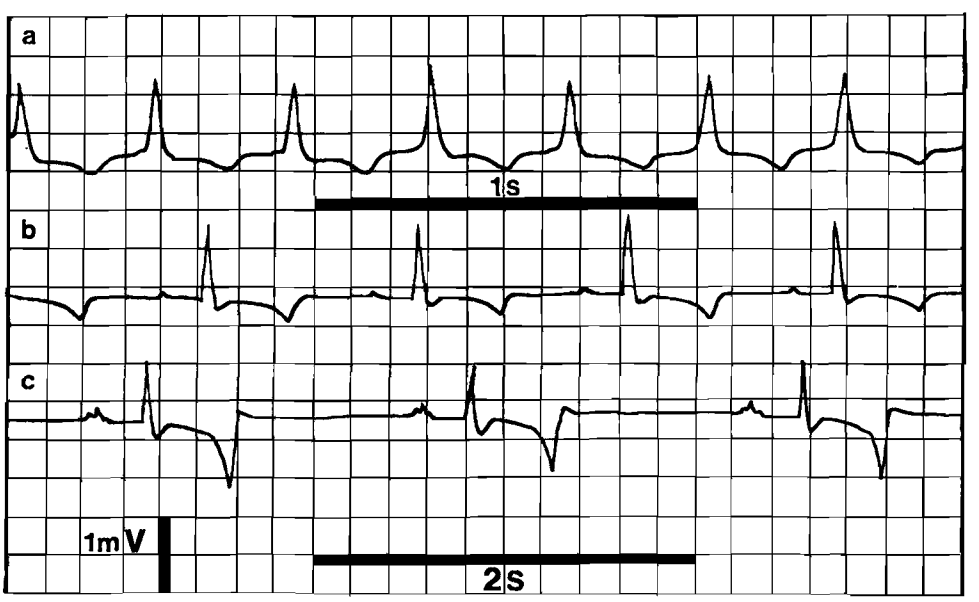

Fig. 1 : Tracings of the electrocardiagram (Lead I) recorded prior to treatment (a). The absence of P waves with QRS and T complexes of normal duration and amplitude is typical of supraventricular tachycardia. The heart rate was approximately 160 beats per min. (b). Tracings of the electrocardiogram recorded approximately 80 min after the oral dosing of 5 g of quinidine sulphate. The tracing shows the presence of a sinus rhythm at a heart rate of approximately 54 beats per min. (c). Tracings of the electrocardiogram recorded approximately 8 weeks after treatment. The tracing shows the presence of a sinus rhythm at a heart rate of approximately 34 beats per min.

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waves. The heart rate was 158 beats per min (Fig. 1a). Based on the above findings, a tentative diagnosis of tachyarrhythmia (supraventricular or ventricular) due to primary heart disease was made and therapy was aimed at the correction of the arrhythmia using antiarrhythmic agents.

Initial supportive therapy consisted of 6 g of procaine penicillin G (Procaine Penicillin, Milvet Ethicals), 1,4 g of etamiphylline camsylate (Millophylline, Centaur Labs) and 100 mg of prednisolone sodium succinate (Solu-Delta Cortef, Upjohn). This therapy was administered following the initial clinical examination and just prior to the transportation of the horse to the private practitioner's hospital.

Once the diagnosis of a tachyarrhythmia was made, treatment was attempted by adding 300 mg of lignocaine hydrochloride (Lignocaine, Centaur Labs) solution to 500 ml of normal saline solution (Sabax). This mixture was infused intravenously as rapidly as possible. This attempt at converting the arrhythmia was unsuccessful and thus a further 240 mg of lignocaine hydrochloride was administered by the same route approximately 10 min after the first dose. This attempt at correcting the arrhythmia was also unsuccessful.

Approximately 15 min after the second dose of lignocaine hydrochloride a 5 g dose of quinidine sulphate (Quinidine Sulphate, Roche Labs) was administered to the horse. This drug was administered by crushing quinidine sulphate tablets and then suspending the powder in water. The suspension was then administered to the horse via a nasogastric tube. Within 1 h of the administration of this drug the cardiac rate had dropped to 100 beats per min. Twenty min later (80 min after dosage of quinidine) an electrocardiogram was recorded and it was found that the arrhythmia had been converted to sinus rhythm and the heart rate was approximately 54 beats per min (Fig 1b). Three further 5 g doses of quinidine sulphate were given to the horse at 2, 4 and 10 h after the initial dose.

Antimicrobial therapy was continued for 5 d following the treatment with antiarrhythmic agents. Bronchodilator (1,4 g etamiphylline camsylate (Millophylline, Centaur Labs) subcutaneously once daily for 5 d) and diuretic agents (500 and 250 mg of furosemide (Lasix, Hoechst Pharmaceuticals) intravenously on the first and second day of etamiphylline camsylate therapy, respectively) were administered to the horse when referred bronchial sounds were heard bilaterally on auscultation of the lung fields 24 h after the last dose of quinidine sulphate was administered. Therapy was terminated 6 d after the horse was admitted to hospital and the horse remained hospitalised for a further 8 d. Following discharge, the horse was allowed stable rest for 6 weeks before being examined for cardiovascular soundness. All the indices of cardiac function investigated during this examination were within normal limits. The electrocardiogram (Fig. 1c) showed the presence of sinus rhythm at a rate of approximately 34 beats per min. Based on these findings the horse was allowed to return to training and has since returned to racing.

DISCUSSION

A definitive diagnosis of cardiac arrhythmia should be based on the careful analysis of an EKG of good quality. Many different electrocardiographic lead systems have been used for the diagnosis of cardiac arrhythmia in horses[5]. As there seem to be no particular advantages or disadvantages associated with the use of any single lead system, a system that produces well-formed and easily discernible complexes should be chosen. Since the impluse that generates a supraventricular arrhythmia originates in the specialised atrioventricular (AV) conduction tissues, they produce a narrow, relatively normal-appearing QRS complex. The initiating impulses of ventricular arrhythmias originate from aberrant ventricular myocardial tissue and thus they are conducted abnormally and more slowly, resulting in the production of QRS complexes of abnormal shape and duration. The T waves associated with QRS complexes of ventricular origin may also be aberrant[3]. In the case reported, the QRS and T complexes were of normal duration and amplitude and thus it is most probable that the tachyarrhythmia was of supraventricular origin.

The aetiology of cardiac arrhythmias is varied. Secondary causes of arrhythmia are numerous. Thus the data base of any case presenting with a suspected arrhythmia should also include history of current drug therapy, measurements of haemodynamic variables and a complete haematological examination[5 6]. Blood should be analysed for serum electrolytes, serum enzyme activities, blood gas tensions and acid-base balance. Primary therapy should be directed at correcting any metabolic abnormalities if the above data suggest that the arrhythmia may be secondary in nature[1 6]. If signs of cardiac failure are present, the primary therapy should be aimed at increasing myocardial performance and reducing the peripheral effects of the myocardial failure[6 7]. As there appeared to be no secondary factors causing the arrhythmia in this case and signs of myocarditis (elevated serum enzyme activities) without evidence of cardiac failure were present, this horse was treated for a primary arrhythmia.

The efficacy of antiarrhythmic agents is varied. Recommended doses[6 7] of lignocaine hydrochloride vary from 0,5 to 4,0 mg kg $^{-1}$. At present there is no pharmacokinetic data to support the dosage regimens recommended for lignocaine hydrochloride in horses. The recommended dose of quinidine also varies widely. Dosages of 20 mg kg^{-1} of quinidine sulphate administered every 2 h[6 7], 4 h[3] and 8 h[1] have been recommended. Based on pharmacokinetic data, a dosage rate of 20 mg kg $^{-1}$ of quinidine sulphate given orally every 2 h until a total dose of 60 g has been administered seems most appropriate[7].

Presently quinidine is the most widely and successfully used antiarrhythmic agent in horses. This is probably due to the fact that quinidine is effective for the therapy of both supraventricular and ventricular arrhythmias. Its popularity can also be ascribed in part to the fact that quinidine is the antiarrhythmic agent that has been most extensively studied in horses. Quinidine is thus the antiarrhythmic agent indicated for the treatment of all tachyarrhythmias in horses, except possibly ventricular tachycardia in animals under anaesthesia, where lignocaine hycrochloride is more suitable because of its rapid effect[6]. In the case reported, the fact that the horse did not respond to treatment with lignocaine hydrochloride provides further evidence that the tachyarrhythmia was probably of supraventricular origin.

Tachyarrhythmias with a rate in excess of 90 beats per min are considered life-threatening and if the arrhythmia is corrected, the prognosis for the retention of previous performance levels is guarded[1]. The response to therapy in the case reported here was dramatic and the horse returned to its previous performance level. However this response should be viewed as an exception, rather than as a typical response.

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DIE KARIOTIPERING VAN DIE LEEU (*PANTHERA LEO*)

M E GELDENHUYS*

ABSTRACT

A short description of the chromosome analysis of the lion, *Panthera leo*, is given. The chromosome number was found to be 38. The chromosomes can be divided into six groups, which consist of submetacentric, metacentric and acrocentric chromosomes.

Key words: Lion *Panthera leo*, chromosomes, karyotyping

Geldenhuys, M.E. Karyotyping of the lion (*Panthera leo*). *Journal of the South African Veterinary Association* (1989) 60 No. 1, 48-49 (Afr) Department of Zootechnology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INLEIDING

In Suid-Afrika is daar geen werk gepubliseer oor die bepaling van die chromosoomgetal en die kariotipering van die leeu, (*Panthera leo*), nie. In verwante werke is geen bandtegnieke gebruik nie[3]. Die projek is aangepak om vir die eerste keer bandtegnieke op die chromosome van leeus toe te pas.

Bloed is van 3 leeus, 'n mannetjie en 2 wyfies, in die Umfoloziwildreservaat, Natal, verkry. Die diere was in 'n baie goeie toestand; een wyfie was dragtig.

Twintig ml bloed is vanuit die *vena cephalica* in geheparineerde bloeibuise versamel. Dieselfde tegniek is toegepas soos by die bepaling van chromosome in die mens, nl. dat van verskillende mediums gebruik gemaak word om witbloedselle te kweek. Hierdie kultuurmediums bevat mengsels van verskeie aminosure, vitamines en gebufferde soute. Drie mediums is gebruik: Medium 199 (modified) (Flow Laboratories); Medium F-10 (HAM) (Gibeo Laboratories) en RPMI Mediums 1640. (Gibeo Laboratories). Vier milliliter van die medium is in 'n digsluitende Falconbuis geplaas; 1 ml fetale kalfserum is bygevoeg omdat elke medium serumproteïnes benodig om die metabolisme van die kultuur te ondersteun. Daarna is 0,1 ml fitohemaglutinien (PHA) bygevoeg om as stimulant vir mitose te dien. Laastens is 0,1-0 3 ml van die gekollekteerde bloed bygevoeg. Die hele proses hierbo beskryf is in 'n laminêre vloeikas uitgevoer om kontaminasie te voorkom.

Die buise is vir 72 h in 'n inkubator by 37°C gelaat. Na verloop van 70 h is 0,5 μg colchicine ml^{-1} kultuur (of 0,1 ml vir elke 5 ml kultuur), by elke monster gevoeg. Die monsters is hierna vir 5 min by 1500 rpm afgeswaai en die helder bostand is verwyder. Daarna is 'n hipotoniese middel, 5 ml van 'n 0,057 M KCl-oplossing wat tot 37°C verwarm is, bygevoeg en is die buis vir 'n verdere 13 min in die inkubator geplaas voordat dit weer afgeswaai is. Die helder bostand is verwyder totdat ongeveer 0,5 ml oorgebly het. 'n Verkoelde fikseermengsel van 3:1 metanol en ysasynsuur is druppel vir druppel bygevoeg, terwyl die buis aanhoudend geskud is, totdat daar 5 ml in die buis was. Die monsters is vir 30 min in 'n yskas geplaas en weer afgeswaai, waarna die helder bostand verwyder is. Die proses is met 5 ml fikseermengsel (2:1) herhaal, en daarna weer met 5 ml fikseermengsel (3:1). Die monsters is oornag by 4°C gelaat en die volgende dag 3 keer met fikseermengsel (3:1) gewas en die laaste helder bostand verwyder sodat ongeveer 1 ml helder vloeistof oorgebly het. Die selle in suspensie is goed gemeng en was toe gereed vir die maak van preparate.

Agt druppels van die selle in suspensie is op vooraf skoongemaakte en gevriesde plaatjies gedrup vanaf verskillende hoogtes bokant die plaatjie. Die preparate is gedroog en oornag by kamertemperatuur gelaat, waarna kleuring toegepas is.

Vir die Tripsien-Giemsa-kleuring is dagoue preparate gebruik, wat oornag in 'n inkubator by 37°C geplaas is. Die preparate is toe vir 10 tot 13 sek in 'n oplossing van 2 ml voorafbereide tripsien en 48 ml fosfaat-gebufferde soutoplossing (PBS) geplaas. Daarna is die preparate afgespoel in 'n oplossing van 48 ml PBS en 2 ml fetale kalfserum. Die volgende stap was om die preparate in suiwer PBS af te spoel, en toe vir 10 tot 15 min in 'n oplossing van 220 ml sörensens-buffer, 10 ml Giemsa en 70 ml gedistilleerde water te plaas, nadat die oplossing eers deur 'n Whatman-filter (nr 1) gefiltreer is. Preparate is een-vir-een geprosesseer om te bepaal wat die beste tyd vir die tripsinering was, en die tydsduur vir die volgende preparate is daarvolgens verkort of verleng. Die preparate is gedroog en onder die mikroskoop beskou. Die preparate met chromosoomverspreidings is rondom die dekglasie met naellak verseël, die beste chromosoomverspreidings is gemerk en gebruik vir latere fotografie en kariotipering. Die kariotipering is gedoen op grond van Hageltorn & Röken se metode van rangskikking[1].

Al 3 mediums wat gebruik is, het goeie resultate gelewer. Daar was 'n voldoende aantal metafases in elke preparaat om tussen 25 en 50 metafases te kon tel, sodat 'n betroubare aanduiding van die chromosoomgetal verkry kon word. Vir elke individu is 50 metafaseselle ondersoek, om die resultaat so betroubaar moontlik te maak.

Die kariotipering van die chromosome volgens die Tripsien-Giemsa-bandtegniek word in Fig. 1 en 2 getoon. Fig. 3 is 'n ongebande kariotipe van die leeumannetjie.

Soos die meeste ander lede van die familie Felidae, besit die leeu 38 chromosome. Hiervan is 36 outosome en 2 geslagschromosome. Die haploïede getal van die spesie is dus n = 19. Daar is wel Felidae met 2n = 36 chromosome, byvoorbeeld *Felis pardalis*[2].

Chromosome kan in 6 groepe verdeel word: Groep A bevat die 3 langste submetasentriese pare; Groep B bevat ook 4 pare submetasentriese chromosome; Groep C bevat die enigste 2 pare metasentriese chromosome; Groep D bestaan uit 4 pare submetasentriese chromosome wat op grond van hul lengtes gekariotipeer is; Groep E bestaan uit 3 pare submetasentriese chromosome wat ook volgens lengte gerangskik is; Groep E bevat die enigste 2 pare akrosentriese chromosome.

In Fig. 1 lyk dit of daar dalk 'n sekondêre konstriksie in die kort arm van chromosoom E2 en moontlik D3 voorkom. By die ander twee figure is hierdie sekondêre konstriksie nie waarneembaar nie.

Die X chromosoom is 'n submetasentriese chromosoom. Daarenteen is die Y chromosoom die kleinste chromosoom in die hele stel en vertoon akrosentries.

Daar bestaan nog leemtes by die kariotipering van die chromosome van die leeu weens gebrekkige literatuur oor die tegnieke van die kariotipering by die Felidae[2 3 4].

Bekende tegnieke moes gewysig word soos die volging van die tripsineringstyd. Die gemiddelde tyd was tussen 10 en 13 s. Medium TC 199 het effens dikker chromosome gelewer as die ander twee mediums, met presies dieselfde tripsineringstyd.

Dit wil voorkom of die tegniek wat by die mens van toepassing is, met slegs enkele veranderinge veral wat betref die kleuringstegnieke, by hierdie spesie van die Felidae toegepas kan word. Kleuringsmetodes kan sekerlik nog verbeter word om bande duideliker te identifiseer.

Om 'n meer volledige navorsingswerk oor leeus te kan uitvoer moet daar met meer individue gewerk word as dié wat in hierdie studie gebruik is.

ERKENNINGS

Dr J. Flamand van die Umfoloziwildreservaat in Natal word bedank vir sy belangstelling en samewerking.

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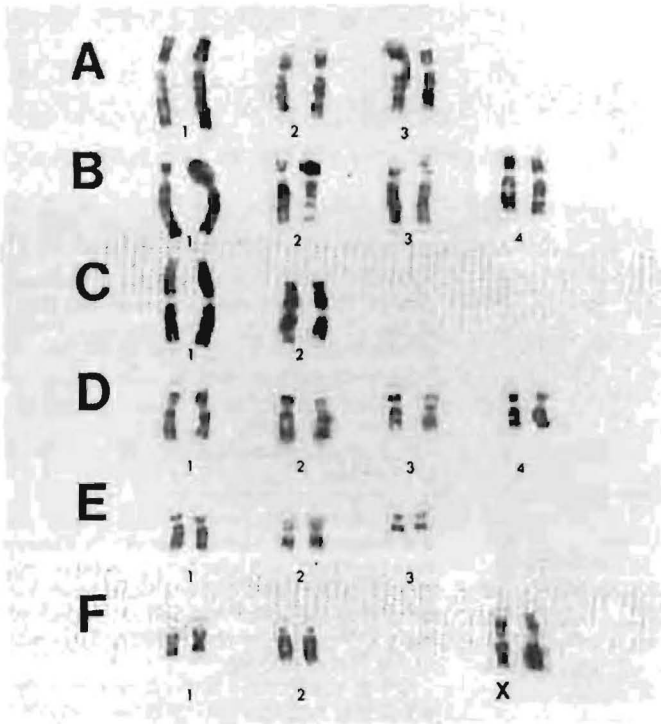

Fig. 1: Kariotipe van 'n leeuwyfie uit die Umfoloziwildreservaat, Natal (Giemsa-bandtegniek)

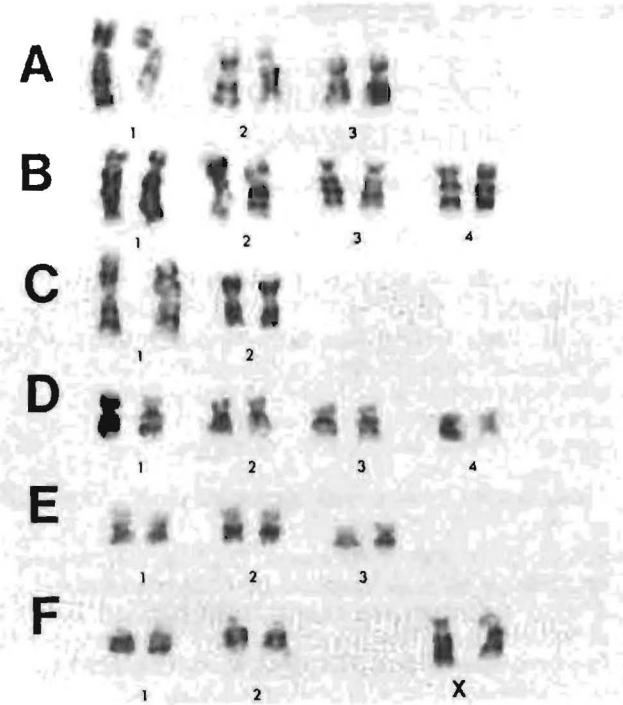

Fig. 2: Kariotipe van 'n leeuwyfie uit die Umfoloziwildreservaat, Natal (Giemsa-bandtegniek)

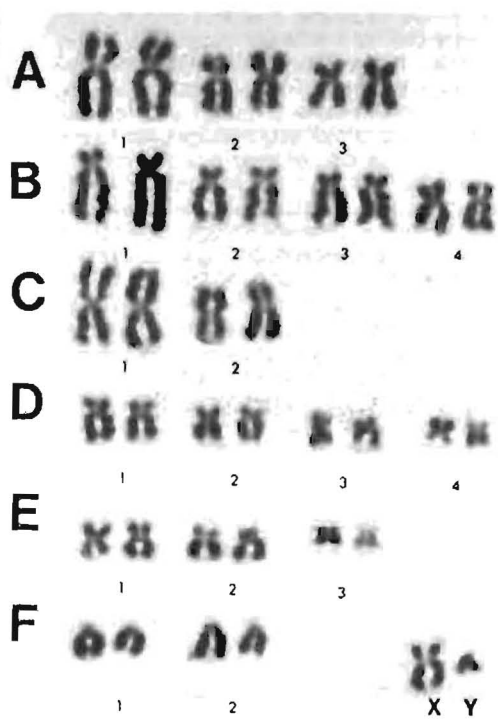

Fig. 3: Ongebande kariotipe van 'n leeumannetjie uit die Umfoloziwildreservaat, Natal

A SEROLOGICAL SURVEY OF BOVINE BRUCELLOSIS IN FOUR DISTRICTS OF BOPHUTHATSWANA

C.J. BOTHA* and C.C. WILLIAMSON**

ABSTRACT

Brucellosis testing was carried out in the Moretele and Odi districts of Bophuthatswana. The cattle tested were heifers and cows over 18 months of age (n = 3 374) which grazed on communal tribal lands under extensive ranching conditions. Fifty (1,48%) animals were tested positive with a CFT titre of 30iu ml^{-1} or higher.

Key words: Bovine brucellosis, serology, communal grazing

Botha C.J. and Williamson C.C. A serological survey of bovine brucellosis in four districts of Bophuthatswana. *Journal of the South African Veterinary Association* **(1989) 60 No. 1, 50 (En.) Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110, Onderstepoort, Republic of South Africa.**

During the compulsory annual anthrax inoculation campaigns of 1985, 1986 and 1987, brucellosis testing was carried out in the Moretele and Odi districts of Bophuthatswana to establish whether the disease was wide-spread in these areas and also to educate the farmers concerning the disease.

The cattle tested were heifers and cows over 18 months of age which grazed on communal tribal lands under extensive ranching conditions. The animals were bled from the tail when the stock owners presented them for their annual anthrax vaccination. Different villages or towns, more or less evenly spaced, with workable crush pens, were selected in the rural areas of Moretele 1 and 2 and Odi 1 and 2 districts of Bophuthatswana. In each village cows and heifers belonging to a number of different stock owners were tested.

The sera were submitted to the Veterinary Research Institute, Onderstepoort for brucellosis testing. The Rose Bengal test (RBT) and the microtitre serum agglutination test (M-SAT)[3] were used as screening tests.

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All sera positive on the RBT or higher than 64iu ml^{-1} on the M-SAT were subjected to the complement fixation test (CFT)[1]. All samples with a titre of 30iu ml^{-1} or higher on the CFT were regarded as positive[2].

In the Moretele 1 district 915 females were bled at 9 different villages (36-150/village). The percentage positive animals ranged from 0% (at 7 villages) to 2,25%.

In the Moretele 2 district 535 females were bled at 6 different villages (44-104/villages). The percentage positive animals ranged from 0% (at 4 villages) to 9,09%.

In the Odi 1 district 1 410 females were bled at 14 different villages (59-150/village). The percentage positive animals ranged from 0% (at 3 villages) to 4,58%.

In die Odi 2 district 514 females were bled at 6 different villages (48-100/village). The percentage positive animals ranged from 0% (at 3 villages) to 4,12%.

There were 3 374 female animals tested in these districts, of which 50 (1,48%) were regarded as positive with a CFT titre of 30iu ml^{-1} or higher.

Although only a small percentage (4,6%) of the total number (n = 72 244) of cattle in the Moretele and Odi districts were tested, it must be emphasised that a large proportion of the herds consist of bulls, oxen and animals younger than 18 months.

Private and trust farms were not included in the survey, which was limited to animals on communal grazing.

The prevalence of brucellosis in animals kept under communal grazing conditions was considerably lower than expected. On some private farms in the districts, where all the female animals (over 18 months of age) were bled as part of a herd diagnostic scheme, the percentage serological positive cases ranged from 0 to 30% (personal observation). The low prevalence of brucellosis in animals kept under communal grazing conditions could in part be due to the annual compulsory Brucella S19 vaccination of heifers. Seven thousand nine hundred and twenty two heifers in the Odi and Moretele districts were inoculated during 1985-1987. (Report of the Department of Agriculture and Forestry, Republic of Bophuthatswana).

Not all susceptible animals are inoculated due to negligence, ignorance and inefficient crush pens. An estimated 60% of heifers between the ages of 3 and 11 months are inoculated annually.

Although individual groups are kraaled at night and the communal herd may concentrate at drinking-places, factors limiting the spread of the disease could be the extensive farming methods, the dry climate and arid areas where these cattle are grazed.

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BOVINE TRICHOMONIASIS IN THE NORTH WESTERN CAPE PROVINCE, WESTERN TRANSVAAL AND THE ORANGE FREE STATE

J A ERASMUS, J A L DE WET, H E VAN DER MERWE, G C J PIENAAR

ABSTRACT:
In bulls (n = 2 437) examined by preputial sheath washes, 7,1% were found to be positive for trichomoniasis. The incidence varied from one area to the other, as well as between breeds.

Key words: bovine, trichomoniasis, South Africa

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In the South African context, trichomoniasis has been regarded as one of the less important venereal causes of infertility in bovines[4]. In contrast, Schmidt-Dumont[5] indicated that 6,2% of bulls tested in South West Africa, were infected with this disease. Retief[4], investigating bovine infertility in the Thabazimbi district in the Republic of South Africa, found 1 out of 9 herds (n = 45) to be infected with the *Tritrichomonas foetus* organism and a variation of the incidence between different breeds of bulls.

In order to determine the incidence of trichomoniasis in beef breed bulls in the north western Cape Province, the western Transvaal and the Orange Free State, sheath wash samples from bulls were examined.

Preputial sheath washings of bulls were taken in phosphate buffer (0,1 M, pH = 7,2-7,3) as previously described[6]. Immediately after collection, each sample was placed into an insulated box, capable of maintaining a temperature varying between 4 to 8°C. All samples were processed in the laboratory within 6 h after collection.

After centrifuging each sample for 10 min at 1 200 g, all but approximately 1 ml of the supernatant fluid was discarded. The presence of infection was evaluated by direct microscopic examination of the suspension obtained, by mixing the centrifugate and the remaining supernatent, as well as by culture of a few drops of the same suspension in modified Plastridge medium[3]. Samples from each culture were microscopically examined after 24 h and again after 96 h incubation at 29-30°C.

A summary of the relevant results is given in Tables 1-3.

The incidence of trichomoniasis varied from 0,9% in the southern districts of the Orange Free State to 10,4% in the north western Cape Province (Table 1). This overall incidence of 7,1% compares well with the reported 6,2% for bulls in South West Africa[5]. When comparing the incidence of infection within breeds, 13,3% of all Simmentaler bulls tested, were carriers of *Tritrichomonas foetus* organisms. This is in contrast to Afrikaners, where all the relevant bulls tested negative for this infection (Table 2). Marked variations were also encountered (Table 3) in the degree of infection when comparing breeds, such as Bonsmaras, Brahmans and Simmentalers in different parts of the country.

In the male, trichomonads are found only on the penis, in the prepuce or in the an-

Table 1: **Trichomoniasis in different areas of the country**

Area	Number of bulls Tested Positive	Percentage positive
Orange Free State (eastern parts)	359	13	3,6
Orange Free State (southern parts)	346	3	0,9
north western Cape Province	1 254	128	10,2
western Transvaal	478	28	5,9
Total	2 437	172	7,1

Table 2: **Trichomoniasis amongst different beef breed bulls**

Breed	Number of Bulls Tested Positive	Percentage positive
Aberdeen Angus	103	6	5,8
Afrikaner	141	0	0
Bonsmara	545	54	9,9
Brahman	493	21	4,2
Charolais	51	5	10
Drankensberger	60	6	10
Hereford	253	12	4,7
Santa Gertrudis	198	7	3,5
Simmentaler	355	47	13,3
South Devon	57	1	2
Sussex	107	3	2,8
Others*	74	10	13,5
Total	2 437	172	7,1

* Numbers of individual breeds too small

Table 3: **Incidence of trichomoniasis in Bonsmara, Brahman and Simmentaler bulls in various parts of the country**

Area	Breed of bull		
	Bonsmara	Brahman	Simentaler
Orange Free State (eastern parts)	0/38 0%	7/62 11%	0/42 0%
Orange Free State (southern parts)	0/65 0%	0/28 0%	1/34 3%
north western Cape Province	45/360 12,5%	12/268 4,4%	45/234 19%
western Transvaal	9/82 11%	2/135 1,4%	1/45 2%

terior urethral orifice[2]. Empirical evidence further indicates that, once exposed, bulls aged 4 years and over, tend to become permanent carriers, whereas younger bulls either spontaneously recover or do not contract the disease at all[1]. Variation in the rate of infection within breeds during this survey, can be partially explained by the fact that all possible age groups were covered. As obvious variations in the incidence of infection within a particular breed in different areas was noted, basic managemental factors may be the main reason for different rates of infection noted between bulls from different breeds. The fact that all Afrikaner bulls tested negative however, may justify further investigation into possible breed resistance to trichomoniasis.

ACKNOWLEDGEMENTS

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TEXTBOOK OF VETERINARY ANATOMY

DYCE, SACK, WENSING

1st Edn. W B Saunders Company, Philadelphia. London, Toronto. 1987. pp XI and 820, illustrations 905, 4 colour plates, 17 tables. Price $52,50. (ISBN 0-7216-1332-2)

Veterinary anatomy is required to offer both undergraduate and postgraduate students in a professional course of studies integrated insight into and comprehension and appreciation as well as certain detailed knowledge of structure as a rational basis for understanding function. The publication in the English language of a textbook of comparative veterinary anatomy is, therefore, a major event since the only other one available, has serious shortcomings. The title is misleading (Introduction to Veterinary Anatomy would have been more appropriate) and the reader is soon disappointed.

This book emanates from some of the leading schools in Europe, the United Kingdom and the U.S.A. Although the authors clearly state that it is intended to meet the needs of the veterinary student (evidently beginners!) the paucity of systematic detail is most disturbing. The subject matter is dealt with cursorily. It is repeatedly stated that there are many species differences with regard to certain structures, but that they are however not important. To dwell on these matters would, therefore, be a waste of time, anyway.

If this is what these schools expect of undergraduate students, the reviewer shudders to think what they expect of postgraduate students in the field of gross anatomy. This trend in a modern textbook foreshadows the complete demise of veterinary anatomy in the English speaking world, not to mention the proud tradition of teaching and research in this basic morphological discipline.

The first ten chapters are devoted to a general description of the body systems based on the dog but with references to the other domesticated animals. The basic principles of mammalian structure and function are presented admirably and the value of this textbook is to be found in the chapters dealing with this material. The remaining 29 chapters are devoted to more specific information of applied and clinical interest with regard to the dog (and cat), horse, ox (and small ruminants) and pig. For each species the regions are described in the same sequence, viz. the head and ventral neck, the neck, back and vertebral column; the thorax; the abdomen; the pelvis and reproductive organs; the forelimb; the hindlimb. For the casual reader the continual cross references between general and special chapters are tiring and irritating. For information on the front limb one has to read the general chapters on the locomotor, nervous and cardiovascular systems, the common integument and the special chapter on the forelimb. The illustrations are scattered amongst these chapters, e.g. a number of blood vessels of the front limb are illustrated in the chapter on the cardiovascular system while others are to be found in the chapter on the forelimb. The book is concluded as is the custom with a chapter on avian anatomy.

The authors lay stress on functional, practical and clinical aspects, surface and radiological anatomy. The tables on the estimation of foetal age are valuable and placentation is described and illustrated in terms of each species. The growth and cyclical changes in the reproductive organs of the mare are described as well as the parturient and puerperal changes in the reproductive organs of the cow. Descriptions of the developmental anatomy of the major systems are valuable innovations.

The book is profusely illustrated with good quality sketches. There are 4 colour plates depicting the placentas of the pig, ox, dog and cat; the fundus of the cat, horse and ox; the oropharynx of the dog and cat; the eye of the dog. In a text of 797 pages there are 905 figures, the majority of which illustrate structures from different angles. The authors have avoided over-labelling the sketches making it a pleasant experience to examine them.

The following errors were noticed:

p 93	The last phrase in first column repeated at beginning of second column.
p 167	Fig. 5-11/6. Paramesonephric incorrectly spelt.
p 190	Par. 1. The glans penis formed over distal end of corpus cavernosum, not the proximal end.
p 239	Fig. 7-39/11. Femoral artery not labelled in sketch.
p 265	First column, second line — overlies spelt incorrectly.
p 300	Fig. 8-64/f. This label for the postganglionic sympathetic to the abdominal organs omitted.
p 395	First column, par. 1. Longissimus dorsi not in accordance with NAV.
p 466	Fig. 18-6/22. Maxillary vein, not nerve.
p 507	Fig. 21-5/1. Label for medial crus of external oblique aponeurosis should be 1".
p 520	Fig. 21-21/13. This label omitted for right kidney.
p 541	Second column. Niekerk spelt incorrectly (two entries).
p 614	Fig. 25-26/5. Middle not medial meningeal artery.
p 626	Fig. 27-3/18. The recurrent laryngeal nerve does not arise at the level indicated.
p 631	Fig. 27-8/7. The aortic and intercostal lymph nodes have the same label.
p 770	Fig. 38-3/2. Medial, not middle iliac lymph nodes.

The book is written in fluent style and easily understandable language. It is recommended without reservation for matriculants embarking on a course of veterinary anatomy.

J M W Le Roux

THE PHYSIOLOGY OF REPRODUCTION

ERNST KNOBIL and JIMMY D NEILL

Published by Raven Press, Ltd. New York, 1988 Vol 1 p xx and 1390, Vol 2 pp xx and 1023 (1391-2413), numerous illustrations and tables, $362,50 (ISBN 0-88167-281-5)

These volumes are a monumental contribution to our existing knowledge of mammalian reproductive physiology while at the same time clearly indicating those areas in which very little knowledge is currently available. From the foreword and through the various sections, chapters and extensive reference lists, the student and teacher of reproductive physiology will find a vast amount of excellent food for thought.

It is an almost impossible task to do justice to a publication of this scope and extent in a limited review. Suffice it to say that amongst the editors-in-chief, associate editors and 100 contributors to this work, one will find some of the best-known reproductive physiologists, endocrinologists and geneticists of our time. The subject matter has been divided into 5 major sections with a number of chapters constituting the content of each section. The majority of chapters are concluded with either a summary or conclusion and each chapter is ended off with an extensive reference list. Both volumes contain a complete list of contents as well as a complete Subject Index (I 1 — 1 90) which ensures easy reference at any time.

Volume I is divided into 3 main sections covering the gametes, fertilisation and early embryogenesis, male and female reproductive systems and finally the hypothalamus and pituitary. The first section consists of 7 chapters, the first of which describes sex determination and differentiation. This is followed by chapters dealing with the mammalian spermatozoon, mammalian ovum, gamete and zygote transport, mammalian fertilisation, early embryogenesis and the biology of implantation.

The second section of this volume is divided into the female and the male reproductive systems. The first 10 chapters on the female system deal with the embryology of mammalian gonads and ducts, the primate oviduct and endometrium, follicular steroidogenesis, follicular selection, mammalian ovulation, the corpus luteum, nonsteroidal regulators of ovarian function, inhibin, relaxin and ovarian steroid hormone action. The subsection dealing with the male reproductive system includes chapters on sex physiology of eutherian mammals; the anatomy, vasculature, innervation and fluids of the reproductive tract; cytology of the testis; the Sertoli cell; synthesis of testicular steroids; structure and function of the efferent ducts, epididymis and vas deferens; androgen action and the accessory sex tissues; sexual functions of erection, emission and ejaculation.

Volume 1 is concluded with a third section consisting of 8 chapters on the pituitary and hypothalamus. These include a chapter on perspectives and overview followed by chapters dealing with the anatomy of the hypothalamo-hypophyseal complex, the role of neuromediators, immunocytochemistry of GnRH, lactotropes and gonadotropes, chemistry and biosynthesis of gonadotropins, gonadotropin secretion and finally, prolactin secretion.

The second volume consists of two sections dealing with reproductive behaviour and its control and reproductive processes and their control. The former is divided into 5 chapters covering topics such as the physiology of male sexual behaviour, cellular mechanisms of female reproductive behaviour, maternal behaviour and the endocrine basis of communication in reproduction. The section is concluded with a chapter on pheromones and mammalian reproduction. The second section of Volume 2 contains some specific chapters on specific reproductive processes in a variety of mammalian species. These range from puberty and the ovarian cycle of the rat and the ovarian cycle of the rabbit to puberty and neuro-endocrine control of the oestrous cycle in sheep. Also included are chapters on puberty in primates and the menstrual cycle and its neuro-endocrine control. Other chapters in this section deal with rhythms in reproduction, seasonal regulation of mammalian reproduction, recognition and maintenance of pregnancy, immunological and genetic factors affecting pregnancy, placental transport and endocrine function (3 chapters), maternal physiology during pregnancy, parturition, prolactin action, lactation, oxytocin and vasopressin biosynthesis and secretion, milk ejection and a chapter on suckling and gonadotropin secretion. The section is very aptly concluded with a chapter on reproductive senescence.

In the preface of this monumental work the editors express the hope "that this book will be useful to all serious students of reproductive physiology, be they scientists, teachers or physicians". I have no doubt that this will be the case and would like to highly recommend it to its targeted readers including the veterinary and animal science reproductive physiologists.

H M Terblanche

WILDPLAASBESTUUR

J DU P BOTHMA (Redakteur)

J L van Schaik (Edms) Bpk., Pretoria 1988 bl 616, 27 tabelle, 136 illustrasies en 8 kleurfoto's. Prys: R59,50 (ISBN 0 627 01470 4)

Hierdie tweede druk van die eerste uitgawe van Prof Bothma se boek is gepubliseer met slegs enkele wysigings. Die boek is primêr gerig op die wildboer en dek 'n wye verskeidenheid van aspekte soos weiveldbestuur, wildplaasbeplanning, wildsoorte, trofeejag, vervoer en aanhouding van wild. Die publikasie is onontbeerlik vir iemand met 'n belangstelling in wildboerdery of wat aktief betrokke is by die bedryf.

J van Heerden

RECENT DEVELOPMENTS IN RUMINANT NUTRITION 2

HARESIGN and D J A COLE

1st Edn. Butterworths, London. 1988 pp 387, numerous tables and figures; Price . . . (ISBN 0-407-01164-1)

The book consists of 22 chapters all of which review various aspects of recent advances and developments in ruminant nutrition. The book deals mainly with dairy cattle but a few chapters are devoted to beef cattle and sheep nutrition. Although the book is probably largely directed at those scientists actively engaged in ruminant nutrition, the summaries at the end of each chapter contain useful information for the large animal practitioner and the dairyman alike. Both physiological and practical aspects of ruminant nutrition are addressed. Factors affecting milk composition and manipulation thereof is of particular interest. Nutritional practices to improve milk yield should be of special interest to the South African-dairy veterinarian.

I believe this is a valuable contribution to nutritionists and veterinarians alike and should also form part of the library of the veterinary academic involved in production animal science.

S R van Amstel

HAEMOPHILUS PLEUROPNEUMONIAE IN PIGS: A REVIEW

C M VEARY*

ABSTRACT:

Haemophilus (Actinobacillus) pleuropneumoniae is a primary and specific pathogen of the respiratory tract and is an economically important pathogen of pigs. The disease is starting to cause peracute deaths in South Africa and the chronic form leads to deteriorating herd performance. This review highlights various aspects of the taxonomic, antigenic, and drug sensitivity characteristics of the bacterium and the epidemiology, clinical signs, pathology, serology and immunology, detection and diagnosis, differential diagnosis, prevention and control of the disease.

Key words: *Haemophilus (Actinobacillus) pleuropneumoniae*, management, histopathology, respiratory pathogen

C.M. Veary **Haemophilus (Actinobacillus) pleuropneumoniae in pigs: A review.** Journal of the South African Veterinary Association (1989) 60 No. 1, 56-61 (En.) Department of Veterinary Public Health, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

Haemophilus (Actinobacillus) pleuropneumoniae is a primary and specific pathogen of the respiratory tract[2] and is the cause of porcine *Haemophilus* pleuropneumoniae (PHP)[18]. This disease has become increasinlgy important to the pig industry throughout the world and now for the first time in the Republic of South Africa (RSA), is resulting in severe economic loss due to peracute deaths, the high cost of treatment of acutely ill pigs and the insidious nature of the chronic form of the disease which subtly erodes profits through deteriorating herd performance[5 18]. The chronic form in pigs must be seen as a complex with most cases being caused by infectious agents, but strongly influenced by environmental and management factors[17]. Stress associated with the latter reduces the resistance of pigs and in the presence of the aetiological factor, clinical pneumonia can manifest itself. Pigs appear to be most vulnerable at 12 to 15 weeks of age during the growing and finishing phase, but clinical disease is not uncommon in neonates and suckling sows. The key facts listed by Lewis & Schwartz[5] must be repeated here:

- *H. (A.) pleuropneumoniae* is transmitted by aerosol among pigs in close contact;
- The most effective control measure is to keep clean, well-ventilated uncrowded pens;
- Recovered pigs are reservoirs of the infection, especially during transportation; and
- Organisms are often sequestered in poorly vascularised tissues; therefore high levels of antibiotics are necessary in treatment.

To control the disease and exclude transmission of PHP amongst herds, both pig producers and veterinarians would like to be able to accurately assess, at a reasonable cost, whether or not bacteria are present in supply herds. The following tests have been tried, but they have not yet proved to be specific or reliable enough[18]:

- post-mortal examination of dead or slaughtered pigs;
- bacteriological culture;
- serological testing; and
- antigen detection

This review, whilst concentrating specifically on the pathology of porcine *H. (A.) pleuropneumoniae*, will also consider developments in detection and prevention of the disease and highlight the role of meat inspection in herd diagnosis[11 16]

AETIOLOGY

Characteristics of the agent: The genus *Haemophilus* comprises short, Gram-negative, non-motile, non-sporeforming rods that require the presence of one or both of the growth factors, Factor X and Factor V, in the media for optimal growth. Growth factor requirements have been used as a criterion for speciation within the genus[5]. *Haemophilus (A.) pleuropneumoniae* requires Factor V but is Factor X independent. Members of the genus as a whole are pleomorphic rods with a tendency to occur mainly in coccobacillary forms.

Due to its resemblance to haemolytic *Haemophilus* strains of human origin, the causative agent of PHP was called *Haemophilus parahaemolyticus*. Taxonomic studies have differentiated the beta-haemolytic organisms of human origin from the pig isolates and, based on certain biochemical and physiological properties, most researchers refer to the causative agent of the disease as *H. (A.) pleuropneumoniae*, as proposed by Kilian et al. in 1978[10]. *H. (A.) pleuropneumoniae* can be identified and distinguished from other porcine *Haemophilus* pathogens using the following criteria:

1. Gram-negative
2. V factor dependent, but X factor independent
3. Urease-positive*
4. CAMP-positive: ferments mannitol*
5. Beta-haemolytic on 5% calf- or sheep-blood agar

Criteria 3 and 4 in particular distinguish *H. (A.) pleuropneumoniae* from other porcine *Haemophilus* pathogens[5].

H. (A.) pleuropneumoniae grows in small, whitish, opaque colonies (1-2 mm within 48h) on chocolate blood agar. Lewis & Schwartz[5] describe two types of colonies.

i) a rounded, hard, waxy type; sticks to loops
ii) a flatter, soft, glistening type

Encapsulated strains produce iridescent colonies on Levinthal agar. Beta-haemolysis is usually produced on calf- or sheep-blood agar with the haemolysin acting synergistically with the beta-toxin of *Staphylococcus aureus* or *Staphylococcus epidermidis*. This creates a satellite phenomenon due to varying degrees of a positive cyclic adenosine monophosphate reaction[5 16]. The *Staphylococcus* strains are non-haemolytic and supply nicotine-adenine dinucleotide (NAD) required by the *Haemophilus*. A small disk soaked in NAD can be used instead of a perpendicular streaking of the *Staphylococcus* strain.

DNA homology techniques have resulted in a new look at the taxonomy of bacteria, with genetic relatedness requiring further adjustments in bacterial classification. Thus it has been demonstrated that bacteria of the genera *Haemophilus, Pasteurella* and *Actinobacillus* can now be included in one group, the so called HPA-group[10]. It is now known that *H. (A.) pleuropneumoniae* comprises two biovars, Biovar 1 being NAD dependent and Biovar 2 NAD independent (previously known as Pasteurella-like organisms). A change in nomenclature is possible, and for the time being the organism causing porcine *Haemophilus* pleuropneumonia should best be referred to as *H. (A) pleuropneumoniae*.

Having said this, identification of the various strains of *Actinobacillus* needs attention to differentiate between both variants of *H. (A.) pleuropneumoniae* and *Actinobacillus suis* (septicaemia, diarrhoea and arthritis), *Actinobacillus lignieresii* and *Actinobacillus equuli*. The table in this regard is reproduced from Nicolet[10].

The different serovars: Mittal et al.[9] recorded that the antigenic properties of *H. (A.) pleuropneumoniae* have been studied and reported on by several researchers[9 10 11]. There is a great serological heterogeneity in the *H. (A.) pleuropneumoniae* group and serotyping is important in both epizootiological and immunological studies of *H. (A.) pleuropneumoniae* infections. The idenfitication and characterisation of the seroptype-specific antigens are necessary for devising more accurate methods for serotyping and serodiagnosis[9]. Type-specific antigens of *H. (A.) pleuropneumoniae* appear to be associated with capsular material, although the precise cellular location is unclear. Mittal et al.[9] record that serotype-specific antigens seem to follow the water phase in phenol water extraction at 65°C, thus suggesting that they are lipopolysaccharide or polysaccharide in nature — as opposed to some *Bacillus* species, where the capsular substance is composed of polypeptides. Based on these capsular polysaccharides, biovar I of *H. (A.) pleuropneumoniae* can be divided into 9 different serovars. Seven of these have been well established, Serotype 8 has recently been added[11] and one more, Serotype 9, has been proposed (Nielsen 1985)[11]. Three more serotypes have been suggested, bringing the total to 12

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Table 1: **Simplified bacteriological differentiation[10] criteria of *Actinobacillus* species isolated in pigs (V = variable)***

	H. (A.) pleuropneumoniae		*A. suis*	*A. equuli*	*A. lignieresii*
	Biovar 1	Biovar 2			
NAD-requirement	+	–	–	–	–
Hemolysis	+	+	+	-/+	–
Urease	+	+	+	+	+
Aesculin	–	–	+	–	–
CAMP	+	+	+	–	–
Trehalose	–	–	+	+	–
Mannitol	+	+	–	+	+
Raffinose	V	V	–	+	+

* Reprinted from Compendium on Swine Haemophilus Pleuropneumonia

Table 2: **Geographic distribution of the different serovars according to the literature[10] and personal identification. The new Serovar 8 was recently described and the publication on Serovar 9 is in preparation[11]. The underlined figures refer to prevalent serovars***

Country	Serovars	Country	Serovars
Argentina	1	India	ND
Australia	1.7	Italy	2.4.9.
Belgium	3.5.	Japan	2.3
Brazil	5	Rumania	5
Canada	1.2.3.5.7	Sweden	2.3.4
Denmark	2.6.8	Switzerland	2.3.7.9
Finland	2.5	Taiwan	5
France	3.7.9	United Kingdom	2.3.8.9
FRG	2.3.9	USA	1.3.4.5.7
GDR	2.5	Venezuela	7
Holland	5.9	Yugoslavia	2.4

ND = not typed

* Reprinted from Compendium on Swine Haemophilus Pleuropneumonia

(Henton M, Veterinary Research Institute, Onderstepoort, 1987 Personal communication). It is probable that certain serovars share a number of common antigens and that some NAD independent (biovar 2) strains appear to have similar capsular antigens. Table 2[10], shows that the different serovars exhibit certain regional or even national preferences.

Mittal et al.[8 9] found that the ring precipitation test was useful in detecting the soluble antigens of the serotype-specific antigens of *H. (A.) pleuropneumoniae* and that the coagglutination test can be used to measure the antigenicity of both soluble and particulate antigens. Mittal et al.[9] worked on the effect of heat treatment on the surface antigens of *H. (A.) pleuropneumoniae* and recorded that

- most of the potent serotype-specific antigens are capsular in origin;
- most superficially located serotype-specific antigenic sites on the capsular layer may be heat labile and antigenically poor;
- the heating of the cells at 56°C or higher may destroy these sites and reveal new structures that are thermostable and antigenically potent;
- the apparent antigenic heterogeneity of serotype 1 strains based on the presence or absence of these thermostable antigens could be valuable in epidemiological investigations.

In summary, the following facts support the theory that the antigens responsible for the serotype-specificity of *H. (A.) pleuropneumoniae* are either polysaccharides or lipopolysaccharides[9]:

- they can be extracted in saline as well as in phenol
- they can be precipitated by ethyl alcohol
- they can withstand boiling and even autoclaving
- they can be extracted from the lung tissues of infected pigs by boiling in saline.

Vitulence factors: *H. (A.) pleuropneumoniae* is highly pathogenic for pigs by the respiratory route — as proven by several researchers experimentally. From Nicolet[10] we learn that some serovars are more virulent than others; 10^2 bacteria of Serovar 1 produced lesions and 10^4 could even prove lethal, whereas with Serovar 2, 10-100 times more bacteria were required to produce the same effect. All serovars with the possible exception of Serovar 3, must be considered as highly pathogenic for pigs. The NAD independent strains of Biovar 2 appear to be less virulent than those of Biovar 1.

In addition to the capsule involvement in the pathogenesis of the disease, the extent of vascular changes in the lungs and the peracute toxic death, strongly suggests an endotoxic shock. Under Pathogenesis other toxic factors affecting lung macrophages and blood mononuclear cells will be considered, as well as a haemolysin, which may be of importance. The complexity of virulence factors involved in this disease might also involve the production of an IgA-protease.

Susceptibility to antibiotics: In vitro, *H. (A.) pleuropneumoniae* is fairly susceptible to many antibiotics. The activity of some antimicrobial substances is shown in Table 3[15]. Schultz[16] states that the drug of choice must be based on the sensitivity of the organism(s) and the lesions observed during slaughter checks.

EPIDEMIOLOGICAL FEATURES

The following factors were identified by Straw[17] to contribute to the development of pneumonia in pigs:

Type of farm: Fewer pigs on closed farms develop pneumonia than those on open farms. Pigs from different origins[17] influence the subsequent extent of pneumonia in a herd: 34,3% of the farms that bought in pigs had pleuropneumonia-positive herds while only 16% of farms that produced their own pigs were positive. The prevalence of pleuropneumonia in herds was 2,4 times higher in open herds than in closed herds and where stock was purchased from sales, the prevalence of pleuropneumonia was 4,7 times greater than in all other purchase practices. When pigs are purchased from only one source the prevalence is not substantially greater than in pigs in a closed herd. The frequency of restocking must also be taken into consideration: the prevalence of pleuropneumonia is 10,8 times greater in herds which are restocked weekly than in closed herds.

Movement of pigs through facilities: The all-in/all-out system as compared to the continuous addition and removal of animals, has a major influence in reducing the prevalence of pneumonia.

Mixing and sorting of pigs: This usually takes place to standardise sizes of pigs in a group as they grow up and move through a system. It results in fighting until the hierarchy is re-established, which is both stressful and a cause of marked transient immunosuppression[17]. In pig farming, disease is a principal reason why certain pigs fail to grow as rapidly as their contem-

Table 3: **Antimicrobial sensitivities of 50 strains of *H. (A.) pleuropneumoniae*** *

Antibiotics (b)	Number of strains (a) Resistant	Intermediate	Sensitive	% Effective
Ampicillin 2 μg	8	2	40	84%
Chloramphenicol(c)	0	1	49	100%
Erythromycin 2μg	46	3	1	8%
Gentamycin 10μg	1	0	49	98%
Lincomycin 2 μg	50	0	0	0%
Neomycin 30 μg	11	39	0	78%
Penicillin-G 2 U	8	23	19	84%
Penicillin-G 10 U	8	4	38	84%
Streptomycin (d)	28	1	0	3%
Spectinomycin 50	1	1	48	98%
Tetracycline 30 μg	9	18	23	82%
Tiamulin 30 μg	1	8	41	98%
Triple sulfa	41	5	4	18%
Sulfachlorpyrazine	8	12	30	84%
Potentiated sulfa	2	6	42	96%

(a) Classified according to criteria of Bauer et al. (1966) as cited by Schultz[15].

(b) Baltimore Biological Laboratories, Cockeyville, MD

(c) Not approved for use in food animals in the United States

(d) only 29 tested

* Reprinted from Compendium on Swine Haemophilus Pleuropneumonia.

poraries. Mixing thus brings healthy young animals into contact with older, diseased pigs and increases the prevalence of pleuropneumonia. There is less tendency to mixing and sorting in an all-in/all-out rearing system.

Number of animals per building: Organisms that cause pneumonia are spread by aerosol and direct contact[17]. The number of possible disease transmissions increases exponentially with the number of animals according to the formula:

Number of possible disease transmission $= n^2 - n$, where n = the number of animals involved.

Floor space per pig: Densely crowded pigs exhibit a higher prevalence of pneumonia than pigs provided with more space. Researchers report that the average space allotment per pig in pleuropneumonia-positive herds, was 0,73 m^2 compared with 0,92 m^2 in pleuropneumonia-negative herds[17].

Ventilation: The design of a building's ventilation system is important in minimising the occurrence of pneumonia in confined pigs. There is a significant correlation between the air space per pig and the risk of pneumonia[17]. An air flow which is not uniform can increase the occurence of pneumonia and drafts must thus be avoided.

Temperature: When the ambient temperature is below that which is comfortable for an animal, the animal's resistance to bacteria in its respiratory tract, is lowered. Pigs under these circumstances cannot remove bacteria from their respiratory tract and are more prone to developing pneumonia[17]. This is particularly true on uninsulted concrete floors, where heat loss from the pig's body through conduction is associated with higher prevalence of pneumonia[17]. The range between maximum and minimum temperatures to which the pig is exposed in a 24 h period is as important as the ambient temperature. Pneumonia tends to flare up when the range is in excess of 5 to 6°C over a 24 h period[17].

Parasites: The migration of *Ascaris suum* has been shown to enhance lesions of pneumonia in pigs. At slaughter checks an increase in severe ascarid scarring of livers is often accompanied by an increase in lung lesions. Several researchers have noticed a considerable reduction in the frequency of pneumonia after successful anti-ascarid programmes were implemented[17].

Straw[17] summarises all this work by saying that clinically the most important factors in controlling chronic pneumonia are:

- an all-in/all-out animal flow
- a single source for herd additions
- proper animal density
- effective parasite control

Optimise these factors and all other factors contributing to pneumonia will also decrease.

Mode of Transmission: *H. (A.) pleuropneumoniae* is a primary pathogen of the respiratory tract with a high host specificity for pigs. In peracute and acute infections it is found not only as a septicaemia or in pneumonic lesions, but bacteria also spread over mucous membranes and in the mucus where the number can reach up to 10^9 bacteria per mℓ of mucus[10]. One infected pig can shed billions of organisms from its nose during an acute natural infection[15]. In chronically infected pigs, the infectious agent is located mainly in the necrotic lung lesions and/or the tonsils, but infrequently in the nasal cavity[18]. Kume and co-workers[3] in Japan, however, recorded a high percentage of nasal carriers in the healthy pig population in Japan. This confirmed previous work by the same workers in 1984, when they recorded a high percentage of apparently healthy carrier pigs in the normal population.

H. (A.) pleuropneumoniae is transmitted by aerosol amongst pigs in close contact[5] or by direct contact[10]. In view of the massive nasal excretion during the acute phase of the disease, indirect transmission by fomites and farm personnel cannot be overruled. However, humans are not common hosts of this infectious agent and most attempts to isolate bacteria from the nose and throats of farm workers have failed.

The organism is susceptible to dessication, sunlight and other elements and does not survive very long in the environment. Normal disinfection on clean surfaces is very effective in killing it. The fragility of the organism limits its dissemination by mechanical vectors or by air, but protected by nasal discharge and mucus it can survive for a few days and be transmitted until completely dried out. Conditions conducive to aerosolisation facilitate the spread of the disease as will crowding in cold weather.

The incubation period may be quite variable and vary from a few hours to a few days.

Spread and outbreak of the disease: Pigs are the chief vectors and hosts of *H. (A.) pleuropneumoniae*. Recovered survivors are a reservoir of infection and serve as the principle source of the disease dissemination[5]. When a healthy carrier is introduced into a susceptible herd under optimal management conditions, the disease may spread subclinically. Clinical manifestation occurs in association with environmental or management stress and commonly follows transportation of pigs. Because of the remarkably short incubation period, susceptible animals in contact with carriers during transportation can develop acute pleuropneumonia and die before slaughter. An abrupt change in climate is another factor that can precipitate the disease. The increased prevalence of the disease is caused by the organism's gradual increase from a low level in summer to a peak in winter or spring[5].

With time, a herd can acquire a high degree of immunity and the infection remains subclinical and the herd apparently healthy. The herd is now considered to be chronically infected and the manifestation of disease under these conditions depends largely on the management conditions. With the disappearance of colostral immunity, pigs may be seronegative and become susceptible to the disease in a finishing unit.

Geographical distribution: *H. (A.) pleuropneumoniae* has been reported from virtually every country where pig production is industrialised[10]. Modern pig management systems favour the development of this kind of infection. The international relationship of the different serovars (Table 3) indicates transmission of the disease through international exchange of animals.

Pathogenesis: *H. (A.) pleuropneumoniae* organism is a normal resident in the nasal cavity[3]. Kume et al.[3] characterised haemolysin produced from actively growing cells, and in 1986[4] suggested that it may suppress pulmonary macrophages. The cytotoxic effect of the haemolysin may contribute to the severity of the lung damage and allows the persistence or proliferation of the organism in the necrotic lung[3]. Lewis & Schwartz[5] support this, saying that *H. (A.) pleuropneumoniae* produces heat labile

and heat stable substances that are toxic to alveolar macrphages and permits multiplication of bacteria instead of clearance. In a sequential study of lesion development in experimental *H. (A.) pleuropneumoniae*, Ligget & Harrison[6] refer to a cytotoxic exotoxin which must be considered as a virulence factor which may decrease phagocytosis and damage phagocytic cells, resulting in the release of potent cellular enzymes. The same authors describe a predominance of neutrophils in alveolar exudates at three and six hours after exposure which they consider a significant finding indicating a major role for this cell type.

The acute gross pulmonary lesions resemble infarcts and have been described as infarct-like. Vasculitis (thrombosis and haemorrhage) tends to support an infarctive origin. Lewis & Schwartz[5] believe that the thrombosis of medium-sized blood vessels of the lungs is indicative of the activity of an endotoxin. Lesions have been associated with endotoxic shock. Ligget & Harrison[6] believe that the vascular damage may be mediated by neutrophils by virtue of toxic oxidant products and enzymes causing injury to pulmonary endothelial cells and pneumocytes. The nature of the lesions in the lung, i.e. localised areas of purulent inflammation and necrosis surrounded by dense bands of inflammatory cells and the formation of an outer layer of granulation tissue, are consistent with the pathogenesis of an abscess[6].

Failure to phagocytose *H. (A.) pleuropneumoniae* organisms may be due to encapsulation, cytotoxins or both.

CLINICAL SIGNS

The disease occurs in three forms: peracute, acute and chronic.

In a susceptible herd the onset is sudden and the spread rapid. Fatalities occur in all age groups.

First signs are a sudden loss of appetite and a high temperature. Some individuals die without showing clinical signs. In rapidly fatal cases there is cyanosis of the skin and mucous membranes, dyspnoea and a moist suppressed cough with a tendency in some pigs to vomit[11]. Rapid, open-mouth breathing may be observed and the pig is depressed and assumes a sitting posture. Epistaxis or a copious discharge of a blood-tinged foam from both the mouth and nostrils, signals death. Lewis & Schwartz[5] refer to a unique feature being the lack of excessive respiratory effort. Death usually occurs within 24 h of the onset of the disease. Mortality in peracute cases is very high: 80-100%. In neonatal pigs the septicaemia is usually 100% fatal and nervous disturbances may be a feature.

The first 4 d are the most critical. Pigs are depressed and reluctant to move because of pain. The duration of the illness might be as long as 5 weeks. During this time intermittant signs of respiratory distress and short suppressed coughing on movement are observed.

Chronically affected animals display no characteristic clinical sings and act as carriers of the organism. Food intake and growth are depressed and there may be a chronic cough.

PATHOLOGY

Gross pathology: When first described by Olander [12] the disease manifested itself as a septicaemia and accompanying fibrinous arthritis, fibrinous pleuropneumonia and a fibrinopurulent meningoencephalitis. Since then most researchers have described additional characteristic pulmonary lesions. The lesions vary according to the duration of the disease. Olander[15] describes the lesions of the peracute infection:

- blood-tinged froth exuding from nares and filling the upper respiratory and tubular air passages
- lungs distended by blood-tinged, oedematous fluid filling alveolar air spaces and interstitial tissues throughout the lungs
- mucosa of air passges is generally bright red
- focal mucosal thickening as a result of oedema and haemorrhage
- lungs may be markedly congested with petechial haemorrhages or may be a uniform deep red colour. A mottled appearance may be caused by multilobular foci of haemorrhage. The latter may be either uni- or bilateral and are more frequently found in the dorsal and hilar regions of the lungs. Pleural surfaces overlying such areas are dull. The lesions may be sub-lobar or involve entire lobes or even the entire lung. Blood diffuses across septae into adjacent lobules and the pleura. Definite demarcation of the haemorrhagic focus is not always clear, but well circumscribed necrotic haemorrhagic lesions may also be found
- hydrothorax and hydropericardium (sero-sanguineous) is common and marked.

Liggett & Harrison[6] studied the effect of *H. (A.) pleuropneumoniae* on the pig over a time span ranging from 3 h to 7 d and the gross and microscopic pulmonary diagnoses following aerogenous infection are reflected below.

If pigs die in acute respiratory distress the post-mortem reveals bronchopneumonia ranging from fibrinous through fibrinohaemorrhagic to fibrinonecrotic exudate. Consolidation occurs unilaterally or bilaterally and is of varying sizes, distribution and extent. These areas are similar in distribution to the haemorrhagic lesions seen in peracute deaths. The affected areas are mottled due to the presence of areas of bright red hyperaemia, yellowish-brown fibrinous exudation and necrosis and haemorrhage ranging in colour from dark red to black. The interlobular septa are thickened and fibrin is usually present on the pleural surfaces, causing the lungs to frequently adhere to the thoracic wall. The adhesions are often separated by pockets of serous fluid[12].

Pigs that survive the acute infection (longer than 4 or 5 d), have characteristic lesions. They are up to 100 mm in diameter, irregular necrotic foci in the dorsal or hilar region. The lesions are encapsulated. The extent of the lesion usually equates with the severity of the clinical disease. With recovery, complete resolution of the lung lesions is possible, but, more than likely, small necrotic sequestra persist, accounting largely for the carrier state of the disease. In a few cases a massive pyothorax may result from extensive liquefactive necrosis. In these the entire thoracic cavity is affected by a copious, fibrinous reaction and organising pleural adhesions.

Histopathology:

Peracute: Lungs manifest as a generalised congestion, haemorrhage and oedema. The alveolar walls are thickened and the alveolar spaces filled with red blood cells and/or eosinophilic fibrin-laden, oedematous or proteinaceous fluid. Coccobacillary bacteria can be seen with Giemsa staining of impression smears. The bacteria can be observed in the exudates as early as 12 h after exposure[6]. This coincides with the peak of the logarithmic growth phase and suggests that bacteria grow uninhibited during the peracute phase. Haematology within the first 6 h showed a leukocytosis with a neutrophilia and a moderate left shift. In this phase neutrophils predominate in the alveolar exudates[6]. Consistent with these findings were the enlarged, firm bronchial lymph nodes with neutrophils filling distended sinuses. There was moderate erythrophagia by sinusoidal macrophages, but no necrosis or increase in the number or size of germinal centres.

Only at this stage does the unique character of the cellular infiltrate as described by Lewis & Schwartz[5] become evident, namely absence of neutrophils and an abundance of mononuclear leukocytes. In fact, the majority of the cells are round and elongated and exhibit severe signs of degeneration that preclude identification[6]. Karyolysis, loss of cytoplasmic organisation, decreased or absent pseudopodia and even rupture of cell membranes in some cases are characteristic changes in these cells. These changes seem to indicate inadequate phagocytic activity by macrophages; neutrophil mediated tissue injury is not prevented. The "infiltration" of mononuclear cells takes place along the interlobular septae and the elongated macrophages are orientated in a characteristic swirling pattern[5].

Acute and later phases: Fibrin and lysed red blood cells still fill most of the alveolar spaces, but the zones along the interlobular septae become more prominent. In adjacent viable tissue, alveolar septa are thickened by hyperaemia, oedema and swelling of endothelial, fibroblastic and alveolar epithelial cells. Fibrinous and cellular exudate fills alveolar spaces at the margin of a sequestrum. Thrombi, comprising degenerating and necrotic leukocytes, are present in the partialy disrupted wall and adventitia of pulmonary vessels at the margin of areas of necrosis.

With age, fibrosis occurs and the fibrinous pleural and pericardial exudates undergo organisation.

SEROLOGY AND IMMUNOLOGY

The fact that *H. (A.) pleuropneumoniae* has different serotypes makes serology difficult. Serotyping of isolates is, however, an extremely important step in the control of the disease[13]. The main feature associated with the serological diversity is the impact it has on vaccination. Natural infection results in a high level of cross-protection between serotypes[11]. Vaccination results do not show this tendency. The use of a monovalent vaccine is best. In spite of low sensitivity, Pijoan[13] concludes that the slide agglutination test must be considered the standard test technique for serotyping. He concedes that an advantage of the coagglutination test described by Mittal et al.[8] is that antigen can be detected directly from lung samples. This is true for pigs with acute respiratory problems, chronic problems and apparently healthy carriers.

The immunity against *H. (A.) pleuropneumoniae* is not completely understood. The capsule, endotoxin and free exotoxin appear to be the main virulence antigens. Following natural infection, circulating

Table 4: **Isolation of *A. pleuropneumoniae* from pigs[18]**

Tissue	154	155	156	159	161	162	164	166	167	172
Nasal swab	–[a]	–	–	–	–	–	–	–	–	–
Turbinate	++[c]	–	–	–	–	–	–	–	–	–
Tonsil	–	–	–	–	–	–	–	–	–	–
Distal trachea	–	–	–	–	–	–	–	++	–	–
Proximal trachea	–	–	–	++	–	–	–	–	++	–
Left bronchus	–	–	+++[d]	–	–	–	–	–	–	–
Right bronchus	–	–	+++	–	–	–	+++	–	–	–
Left lung	–	–	–	A[e]	–	++	++	–	–	A–
Right lung	–	A–	–	A+[b]	–	–	–	–	–	A–
Maxillary lymph node	–	–	–	+	–	–	–	–	–	–
Bronchial lymph node	–	++	+	–	–	–	–	–	–	+++
Spleen	–	–	–	–	–	–	–	+	–	–

–[a]	=	No. *A. pleuropneumoniae* isolated; i.e. negative
+[b]	=	1 to 100 CFU/g
++[c]	=	101 to 1000 CFU/g
+++[d]	=	more than 1000 CFU/g
A[e]	=	pulmonary abscess; indicated by A

antibodies develop within 2 weeks. The organism may persist, however, in necrotic pulmonary sequestra and/or in the tonsils of pigs considered to be immune[5]. It is unusual to see clinical signs in chronically infected breeding herds. Passive immunity is conferred upon piglets by immune sows, and acute cases have been observed amongst piglets 3-8 weeks of age when colostral antibodies have declined to low levels[11]. Fenwick et al.[1] found that generally immunisation and serological identification of carrier animals does not slow down the spread of the disease or reduce losses associated with infections. The same authors studied the effect of immunisation with cross-reacting lipopolysaccharide core antigens of *E. coli*, and concluded that this holds promise as a means of increasing non-specific host resistance against Gram-negative infections[1].

DIAGNOSIS

Detection: This has been worked on by Willson et al.[18] whose success from bacteriological culture methods is shown in Table 4. They alert us to certain problems in the detection of *H. (A.) pleuropneumoniae*, namely that

- sequestered organisms in carriers are seldom sampled positively
- *H. (A.) pleuropneumoniae* may be overgrown and killed by other bacteria prior to reaching a laboratory
- the organism is fastidious and requires special culture techniques.

Some observations from this study include:

- that there is no fully reliable method to determine whether or not a pig herd is infected;
- bacteriological culture of nasal swabs indicates a peak prevalence at about 12 weeks of age;
- the bronchial lymph node should be cultured in addition to lung tissue when screening for *H. (A.) pleuropneumoniae*;
- the organism was not isolated from lungs without lesions; and
- many of the organisms survived at least 4 days under various storage conditions under 20°C.

Nielsen[11] listed 4 diagnostic methods for the detection of *H. (A.) pleuropneumoniae* infection in pigs

- clinical signs and the course of the disease in individual pigs and the herd
- post-mortem findings
- isolation of the bacterium from the respiratory tract
- serology

DIFFERENTIAL DIAGNOSIS

a) *Haemophilus parasuis* (Glässer's disease): similar in the initial phases, but Glässer's disease eventually shows the characteristic signs of joint swelling, lameness and nervous disturbances. On post-mortem, a fibrinous polyserositis is evident; lung tissue is rarely involved.

b) *Mycoplasma hyopneumoniae* (Enzootic pneumonia): This organism decreases ciliary function and causes ciliary loss. It is also capable of suppressing the cell-mediated immune response. It produces a persistent cough following a slow development phase and is characterised by firm, plum-coloured areas of consolidation in the cranioventral portions of the lungs.

c) *Pasteurella multocida:* usually a secondary invader with lesions predominantly in the cranio-ventral lobes of the lung. It causes a purulent bronchopneumonia sometimes accompanied by a pericarditis and pleuritis.

d) Vitamin E and selenium deficiency: sudden death syndrome.
Nielsen[11] and Schultz[16] both refer to and emphasise the role of the abattoir in the herd diagnosis of pneumonia of a chronic nature. Schultz[16] says that not only is it a good way to assess a herd's pneumonia problem, but an even better way to monitor the effectiveness of changes in preventive and control programmes.
The haemolysin obtained from the supernatant of *H. (A.) pleuropneumoniae* culture were both cytocidal and had antiphagocytic activities in pulmonary macrophages in the cotton rat, guinea pig and pig[4]. These findings might prove of importance in both the pathogenesis and diagnosis of this disease.

PREVENTION AND CONTROL

The consensus of opinion is that prevention and control is based on

- early clinical and aetiological diagnosis[5 7 11 15]
- therapy and monitoring during therapy
- management practices; emphasis placed on avoidance of over-crowding
- environmental control; emphasis placed on adequate ventilation
- serological testing in an attempt to maintain a disease-free herd in an all-in/all-out system
- vaccination after 5 to 6 weeks of age.

The details will not be further elaborated on in this review.

CONCLUSION

Ross[14] looked at some of the problem areas associated with *H. (A.) pleuropneumoniae*, and whilst progress has been made, many of his conclusions remain thought- provoking:

- although the organism is fastidious, methods of isolation and identification are generally adequate
- improved methods of direct detection of the organism in fresh and formalin-fixed tissue would enhance diagnostic work. The coagglutination test reported by Mittal et al.[8] could prove a useful rapid detection test in infected tissue
- serotyping of strains is not sufficiently accurate to be of practical value in screening pigs prior to herd introduction to new herds. Serological procedures using monoclonal antibodies might facilitate the differentiation of pathogenic from low-virulence or non-pathogenic strains
- the taxonomic status of the organism needs to be finalised — what, if anything, is the significance of the taxon "Minor Group"?
- regarding serodiagnosis, better understanding is required of the relationship between seropositive tests and the actual presence of organisms in the host
- what is the antibody response to the administration of *H. (A.) pleuropneumoniae* bacterins and what is the significance? The carrier state is not eliminated
- the work of Liggett & Harrison[6] has greatly enhanced the understanding of the pathogenesis of the disease, but continued systematic investigations into the pathology and pathogenesis are required. The immunogenic or deleterious effects of endotoxin, capsular material, cytotoxin and other factors will have to be conclusively determined
- vaccination reduces loss of a clinical nature, but does not stop the spread of the disease and has caused substantial loss at the abattoir from granuloma and abscess condemnations
- improved therapy of an infectious disease requires knowledge of the pathogenesis and pathophysiology of the disease. In *H. (A.) pleuropneumoniae* the organisms are deeply seated in poorly vascularised pneumonic abscesses and high concentrations of therapeu-

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tic agents must be administered to achieve optimal blood levels. Even then the response is not always satisfactory and treatment becomes protracted and expensive.

From the pig farmer's point of view the advice of Lewis & Schwartz[5] should be noted. Improve management and keep pigs in well-ventilated pens, avoiding crowding. Clean and disinfect pens between occupation by various groups of pigs, adopt and all-in/all-out management system and minimise pig movement and sorting.

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SOME HUSBANDRY FACTORS INFLUENCING WEANING STRESSES IN PIGLETS

B T SPENCER* and P G HOWELL**

ABSTRACT

The post-weaning diarrhoea syndrome (PWDS) in piglets is multi-factorial in origin. Numerous managemental, environmental, housing, nutritional, immunological and physiological factors are discussed, being primary factors in the cause of the PWDS. Infectious agents especially *Escherichia coli* are often incriminated as causes of PWDS but are more likely to be opportunistic elements due to faulty management of stressed animals.

Key words: post-weaning, diarrhoea, management, immunology, physiology, diet, piglets

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INTRODUCTION

The post-weaning diarrhoea syndrome in piglets results in scours, poor performance and even mortality. Stability of the bacterial microflora immediately after weaning assists the piglet in surviving the most crucial stage in its life after parturition. Post-weaning diarrhoea is different from neonatal scours and most other scours as the condition is not age-dependent but associated with a particular event, namely weaning[9]. Intestinal villous atrophy and bacterial colonisation occur 3 — 4 d after weaning with partial recovery of the mucosal integrity 4 — 7 d later. The bacterial colonisation is preceded by villous atrophy as indicated by Kenworthy & Allen[7].

The high recovery rate of *Escherichia coli* in weaned piglets with digestive disturbances, implies its continual presence, but as an opportunist to the intestinal changes which occur at weaning, rather than as a primary pathogen[9]. These authors have hypothesised that hypersensitivity to dietary antigens may cause villous atrophy predisposing to colonisation of of the duodenum by *E.coli*.

The post-weaning diarrhoea syndrome is treated differently on different farms for various reasons. The severity of the problem, quality of housing and owner/manager cooperation often determine which control measures should be taken. A broad background knowledge of aspects of housing such as micro-environment and ventilation, as well as nutrition, diseases affecting the piglets, immunology and drug pharmacology, assist in rectifying the problem.

WEANING IN PRACTICE

Weaning is a traumatic event for a piglet which is used to warmth, a dry environment, mainly liquid food and the comfort of the sow. The management of the piglet should be aimed at supplying the correct housing, nutrition and temperature requirements to sustain optimal growth. As the weaning age of piglets is slowly reduced by changing managemental practices, the weaning crisis is forced on less mature animals which cannot adequately cope with the stress.

The weaned piglet's diet is abruptly changed from predominantly liquid (sow's milk) to solid food. The quantity of solid creep-feed that piglets consume before weaning, is normally very small and is influenced by managemental practices, age of weaning and feed palatability. The weaner feed is generally designed to closely resemble sow's milk and aid the adjustment to changed feed constituents. At weaning, the piglet's digestive, physiological and immunological systems are still immature.

THE ROLE OF GASTRIC pH

The rapid growth of the young piglet is assisted by an efficient digestive system. The physiology of digestion in the piglet enhances both digestion and protection through its normal functioning. The gastric pH of the suckling piglet, normally ranging from 2-4, is capable of aiding protein-digestion and serves as a protective mechanism which causes ingested pathogens to be inactivated[14].

The acidic state in the stomach aids the clotting of milk and assists pepsin to digest milk proteins before final assimilation in the small intestine. As the piglet develops, the digestive tract increases in size and produces larger quantities of the enzymes rennin and pepsin which are required to digest milk. This enables the suckling piglet to increase its milk intake. The digestive system also increases the range of enzymes it secretes. These digestive enzymes include amylase, maltase, sucrase and proteinases. As these enzymes are produced in larger quantities, the piglet develops the ability to digest solid feed[15]

Milk has a high digestibility value, in the region of 95%, primarily due to its small particle size and composition, which is suited to the immature digestive system of the piglet. The lactobacilli that inhabit the stomach ferment lactose which serves as an energy source while producing lactic acid that is absorbed and readily utilised by the piglet. The lactic acid is the main acidifying agent in the stomach of the young pig up to about 3 weeks of age. After this age, hydrochloric acid secretions have a greater acidifying action[15].

The acidic state of the stomach limits the multiplication of pathogenic strains of *E.coli* in piglets where the pH is below 3,3[16]. This low pH is regularly attained in the stomach of suckling piglets which initially suckle hourly and at half this frequency after a few weeks. The clotting process of the milk occurs at low pH levels, with consequent limitation on the development of *E.coli*.

Acidifiers have been added to feed and water in an attempt to keep the upper digestive tract acidic and hence limit the growth of pathogens[14]. An improved growth rate and feed conversion efficiency was recorded after the addition of 1% or 2% citric or fumaric acids to rations. This improvement was obtained notwithstanding marginally poorer feed intake. It was postulated that this improvement was achieved because of improved utilisation of nutrients in the weaned piglets on the acidified feeds, as opposed to the controls[2]. Similar work done suggested "that the pig which is under stress after weaning, may benefit from administration of glucogenic, tricarboxylic acid cycle intermediates, such as citric or fumaric acid, which may prevent some tissue wastage resulting from high rates of gluconeogenesis and lipolysis"[3].

When lactic acid is added in the water, or a high bran content feed is fed, a greater decrease in gastric pH is observed than when less fibrous rations are fed and clean water is offered. This lower gastric pH has the effect of reducing the multiplication of pathogenic *E.coli* even further. The gastric pH must be maintained at 3,6 or less to be effective in limiting *E.coli* initiated disturbances. This will result in a lower mortality rate and a better growth rate and thus represents a major economic benefit to the farmer[16].

It is maintained that gastric acidity is important for digestive enzyme function and also serves as an inhibitory agent for bacterial growth. Weaning itself causes disturbances in the normal functioning of the stomach. The simultaneous change of feed allows enteropathogenic strains of *E.coli* to colonise the upper parts of the small intestine where they normally do not occur[14]. After weaning, digestive disturbances occur more freely than prior to weaning because of poorer digestion. Multiplication of pathogenic bacteria takes place more freely with a raised pH. Schulman[14] found that the gastric pH of weaned piglets remained higher than that of unweaned piglets for corresponding periods after a meal. The gastric pH of suckling piglets dropped to under 3,0 within 3 hours after feeding while the gastric pH of weaned piglets was over 4,0. The survival rates of pathogenic *E.coli* are reduced at pH levels of 3,6 and less[16].

IMMUNOLOGICAL MECHANISMS

The immunological system of suckling and weaned piglets is effective if the piglet is reared where management practices compensate for accommodation and nutritional limitations. The piglet is unable

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to receive passive immunity via the placenta but readily absorbs IgA, IgG and IgM from the colostrum. These absorbed immunoglobulins have the ability to protect the piglet against infection, provided the sow was immune. This is best demonstrated by the protection of the piglet against *E.coli* by colostral immunoglobulins in sow's milk[12].

The piglet receives no immunogloblin through the placenta of the sow. The neonate absorbs IgG, IgA and IgM from the colostrum and once protein digestion commences, immunogloblin absorption ceases. The local mucosal antibody system in the piglet ensures that the mucosa is bathed in immunogloblin produced in the lamina propria[12]. Smith[15] speaks of a "special immune protein which is constantly secreted in the milk of the sow, which is neither absorbed through the gut or digested by the gut enzymes, and bathes the cells lining the gut as long as the piglet is suckling". This special protein is either a trypsin inhibitor that protects colostrum from digestion, or more probably, IgA produced by the sow and secreted with the milk[12]. Porter[12] confirmed that the local antibody mechanism recorded as long ago as 1922 by Davies, and Bezredka in 1927 is important in providing protection against invasion through the gut, although the intricasies were only unravelled with the identification of IgA[5].

The sow's milk contains a large quantity of mammary secretory IgA that bathes the epithelium of the upper gastro-intestinal tract. IgA shows a resistance to digestion by enzymes and protects the whole alimentary tract[12]. The alimentary tract is the first system that is seriously challenged by ingested infectious agents. The lamina propria and Peyer's patches respond with the production of IgA and IgM, respectively. The Peyer's patches are extremely active in the young piglet. At weaning, almost equal quantities of IgA and IgM are being produced, yet in the adult pig IgA is the main gastro-intestinal immunoglobulin secretion[11]. Thus it can be assumed that the alimentary immunity of the weaned piglet is too immature to protect it from the challenge of pathogens in its digestive tract. The practice of earlier weaning leaves the piglet with insufficient immunoglublin other than IgM and it is thus less readily able to protect itself.

Secretory IgA is the predominant immunoglobulin in the alimentary, respiratory, urogenital, mammary and salivary systems of mature pigs. This age-related protective mechanism of the piglet is enhanced once IgA predominates over other immunoglobulins.

The immune system of pigs is enhanced by adequate vitamin E and selenium concentrations in body tissues. The piglet is exposed to two critical periods of vitamin E deficiency, they are the neonatal period and the period directly following weaning. The period of neonatal deficiency is minimised if the piglet is able to receive sufficient vitamin E from the colostrum. The piglet must itself ingest and assimilate sufficient vitamin E over the weaning period. "Selenium dynamics indicate the existence of a critical period after weaning"[17]. Vitamin E and selenium are essential for the intra-cellular and extra-cellular activities which maintain cellular integrity. Deficiencies are caused by a host of conditions all related to a breakdown of cellular integrity mainly of the walls of the arterioles of the organs affected.

ENVIRONMENTAL STRESS

Pigs have a definite social status within groups with respect to bodily functions. This feature is seen even in young piglets. The pig in general is very protective of its environment. The fact that boars fight using their shoulder shields as a protective mechanism and that sows and growing pigs fight when they are mixed, is proof of this. This aggressive nature is inherited and is observed even in suckling litter mates where play often degenerates into aggression.

The repeated fighting after weaning leads to irregular feeding and overeating, which results in reduced peristalsis and the multiplication of opportunistic pathogens. The piglet's defence mechanism is also reduced at weaning by certain managemental practices. These practices include regrouping piglets according to size and sex or simply into large groups. This results in social stress on the piglet, which is aggravated by overcrowding, a new environment, mixing, fear and anxiety. The increased level of stress causes the reduction or cessation of gastric activity[15]. This causes the gastric pH to rise, opportunistic pathogens to develop in the intestine and as is often observed, diarrhoea after weaning.

Other problems may develop due to overcrowding or regrouping and these may have an effect on the functioning of the alimentary tract. Altered dunging patterns and tail biting are vices that are often seen in weaners. The changed dunging habit can be caused by physical limitations on the piglet's movement to the dunging area and by its fear of crossing the territories of dominant piglets. Poor ventilation must be considered as one of the most common causes of reversed dunging patterns. This is postulated as being the result of poorly ventilated areas retaining the smell of excreta and encouraging other piglets to use this area as a dunging area.

Changes in humidity can also result in diarrhoea. With naturally ventilated weaner accommodation, which is very common locally, changes in humidity can rapidly occur during summer thundershowers. Research done in Belgium showed that piglets exposed to a humidity change of 10% over a 30 min period, may develop diarrhoea[15]. There is no current explanation for this observation.

The temperatures between the upper critical temperature (UCT) and lower critical temperature (LCT) are the limiting temperatures that have been found best suited for pigs. The range varies depending on size, grouping, housing and growth phase. The overall norm for weaned piglets is 20 — 30°C but as the weaning age is reduced and solid flooring removed, constant temperatures approaching the UCT are necessary. With less capital intensive weaner accommodation, the stockman is taxed to attain the necessary performance. Reduced performance and diarrhoea are more frequent with fluctuations in temperature and as the LCT is approached.

Diurnal temperature fluctuations can initiate diarrhoea. The piglet is stressed when the temperature approaches the LCT where a 2°C change in temperature at piglet level within 30 min will precipitate diarrhoea[15]. This is an increasing problem with the changing housing patterns which do not allow the piglet to adapt to its own micro-environment. Bedded kennels are being replaced by hygienic flat deck systems in semi- and fully ventilation-controlled housing in many modern piggeries throughout the world. The reduced availability of bedding and the striving for reduced labour requirements have thus mitigated against the needs of the piglet.

PHYSIOLOGICAL MECHANISMS

The suckling piglet suckles about every 2 h at weaning age. The digestive tract is conditioned to this frequency of taking in small quantities of milk and almost no solid feed. The weaned piglet is stressed by the removal of the sow and is often moved and grouped with other piglets of similar size and sex and then offered a palatable feed ad libitum. The result is frequent gorging which overtaxes the ability of the digestive tract. This lays the foundation for the incomplete breakdown of feed, with partially digested products being forced further down the tract causing malabsorption and digestive upsets.

The digestive tract of the young piglet is very sensitive to a wide range of stresses. These factors result in a physiological disturbance and what is termed a 'non-infectious diarrhoea'[15]. Small changes in the environment of the piglet can result in a diarrhoea initially of non-infectious origin that later becomes aggravated, following invasion by opportunistic bacteria which are present in the gut or environment.

Reduced peristalsis due to gorging often occurs after weaning. The static ingesta permits the multiplication of bacteria within the lumen. These bacteria can develop in the ingesta or, as with certain strains of *E.coli*, while attached to the mucosa of the lumen[10]. The ability of certain *E.coli* to attach and colonise depends on their pili. This is best demonstrated by the K88 antigen. These colonies of *E.coli* liberate enterotoxins that affect electrolyte and water balances in epithelial cells and result in the symptoms of enterotoxaemic colibacillosis.

Underfeeding which results in gut stasis is as dangerous a trigger mechanism for diarrhoea as overeating. A hungry pig is immediately unhappy and seeks food, comfort, warmth and company. The fellow piglet's excreta are readily available sources of sustenance and thus coprophagia occurs. This is often the result of managemental errors such as overcrowding, grouping uneven size weaners, insufficient feeder space, cold housing and a poor water supply which all contribute to depriving the piglet of feed. The load of infectious agents in the starved, cold and immunologically threatened piglet is immediately high, often high enough to institute an infectious diarrhoea in the piglet.

DIET

Before weaning, the piglet is accustomed to a liquid diet of milk supplied in regular small quantities. Sow's milk which has a high energy content, consists of 80% water, the balance containing 5,4% protein, 8,3% fat and 5,0% lactose. After weaning, clean fresh water is a critical component of the nutrition for the piglet, if it is to develop rapidly. Water is provided by automatic drinkers that are designed to reduce both the loss and contamination of water. Sweeteners are often added to the water supply to stimulate intake. Although the objective is to increase water intake, the sweetener can pass to the lower intestine following overloading of the digestive system. This can result in a diarrhoea, due to the incomplete digestion of the sucrose, lactose or artificial sweetener that is used.[15]

Weaner feeds have a digestibility factor

of 70 to 90% depending on the ingredients. Creep and weaner feed intake can be enhanced by treating the ingredients by cooking, flaking, rolling and acidifying with preservatives which extend its storage life. The final feed intake can be improved by heating, pelleting and flavouring and is influenced by good physical form and the method of presentation.

The ingredients used in weaner feeds can cause digestive upsets and hence hamper efficient growth of the piglets. Sucrose is poorly digested, as it needs to be broken down by the enzyme sucrase which is produced only in limited quantities in the young piglet. Appreciable quantities of sucrase can be produced at 4 weeks of age but as is recorded by Hampson & Kidder[4], significant reductions in digestive enzyme activities occur at 4 to 5 d after weaning. Peak amounts of sucrase are however produced at about 5 months of age. Should sucrose pass to the colon, the osmolarity of the solution in the gut encourages further passage of fluid to the lumen of the colon. This results in a malabsorption type of watery diarrhoea[15].

There is a continuous excretion and absorption of nutrients, minerals, electrolytes and water to and from the lumen of the alimentary tract. Certain areas are known to be nett secretory areas, for example the stomach and small intestine, while the colon is a nett absorption region. The secretion aids the passage of the watery ingesta to the colon where water is reabsorbed. Sucrose, incompletely digested feed and feed breakdown products have a water retention capability that reduces the absorption of water by mature epithelial cells. A volume-instituted diarrhoea results. Bacterial action in the colon on abnormal colonic nutrients produces substances such as lactic acid. These substances irritate the lumen, as well as being osmotically active, thus preventing water absorption and causing further diarrhoea.

Thus, once malabsorption has commenced, the snowball-effect within the colonic lumen aggravates the situation further, until diarrhoea which is difficult to control, results. Dehydration is a serious consequence.

The major problem causing death of the weaned piglet is dehydration[15]. This dehydration is caused by the malabsorption syndrome that reaches its peak 7-10 d after weaning. Water is very necessary for the piglet after weaning, as the feed is changed from a liquid to a solid form. Litter mates accustomed to suckling at the same time cannot all drink water simultaneously from a single watering point.

BACTERIAL FLORA AND INFECTIOUS AGENTS

Numerous bacteria are normally found in the digestive system, living a symbiotic life with the host. The importance of the ecology of the indigenous microflora, motivated studies on piglets to establish the colonisation of their stomachs and small intestines by porcine and human strains of lactobacilli. *Lactobacillus spp.* have the ability to adhere to the stomach epithelium and thereby produce lactic acid[6]. The *Lactobacillus spp.* are the major bacterial flora in animals such as weaned piglets which are on a cereal diet[18].

The normal indigenous microbial species form populations, which are often mixed, in the alimentary tract. They are necessary for the stability of that ecosystem[13]. In recent years there have been attempts to install specific gut flora in the piglet by including *Lactobacillus spp.* as a probiotic feed, the rationale being that these bacteria in the stomach and upper small intestine, promote an acidic environment that hampers the development of pathogenic Gram-negative bacteria[19].

Koopman[8] found that "the microflora present in the intestinal tract of animals and birds will ensure that pathogenic bacteria will not become colonised or will have difficulty in doing so and cannot therefore grow into large populations in the intestinal tract". This phenomenon he termed "colonisation resistance". The microflora of the intestinal tract of healthy animals are in balance and a stability is established within the tract, which is related to specific pH levels of the various regions of the intestine. Disturbances of the microflora and pH disrupt this "stabilisation".

Koopman[8] found that a high load of infection by pathogenic bacteria, poor general resistance, the use of antibiotics, fasting, variation in the diet and stress were all factors which had an adverse effect on the microflora present in the host. He also claimed that age is a factor, with young animals showing disturbances of the intestinal flora more frequently, thus making weaning age a critical factor. Animals are consequently particularly susceptible to intestinal disease immediately after weaning.

This unstable milieu in the digestive tract permits the invasion of the tract by secondary and opportunistic organisms, especially *E.coli*. *E.coli* is often listed as the cause of death, yet it is only acting as an opportunistic bacterium multiplying in the more suitable post-weaning stressed duodenum and jejunum. Other primary and secondary organisms may also affect the digestive tracts of weaners.

The most common enteric infectious diseases affecting weaners include: Transmissable gastro-enteritis, rotavirus, salmonellosis, swine sysentery, campylobacter mucosalis infection and coccidiosis. Some of the pathogens are virulent enough themselves to cause scours or even death. Others act in unison with commensal organisms, while others require a stressed system in which to become effective. Good managemental practices and an enhanced immune status of the weaned piglet will assist in combating these pathogens[1].

CONCLUSIONS

One can therefore conclude that there are a multitude of factors to consider when advice is offered to pig farmers whose weaners are exhibiting a mortality rate above 2% or an increased incidence of poor performance. The PWDS must be studied as an independent case on each farm due to differences in housing, feeding, breeding and management.

The financial returns to the farmer are higher if the weaned piglet can maintain a healthy, optimal growth rate after weaning. Various managemental techniques as well as housing, micro-environment, allocation of space, nutritional and medication practices play a role in achieving this goal.

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EICOSANOIDS: A SHORT REVIEW

J G VAN DER WALT*

ABSTRACT:

The biochemical reactions leading to the formation of eicosanoid compounds are reviewed. Arachidonic acid and similar homologous polyunsaturated acids are shown to be important precursors of these compounds. The partitioning of precursors betweeen the cyclo-oxygenase and lipoxygenase enzyme systems leads to the prostanoid/thromboxane/prostacyclin and leukotriene families of compounds respectively. The synthesis and catabolism of each of these groups are reviewed. The mechanism of action of the prostacyclin group is briefly discussed.

Key words: Review, synthesis, catabolism, eicosanoid, prostaglandin, prostacyclin, thromboxane, leukotriene.

Van der Walt, J.G. **Eicosanoids: a short review.** *Journal of the South African Veterinary Association* **(1989) 60, No. 1, 65-68 Department of Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.**

INTRODUCTION

Monocarboxylic aliphatic acids constitute a large fraction of the total lipid pool, and vary considerably in chain length (from 6 to 24 carbons), degree of saturation (number of ethylene bonds) and isometric configuration (*trans* or *cis*). Despite this wide diversity, animals have the capacity to synthesise most of these structures, requiring only 2 or 3 fatty acids that may therefore be regarded as essential, namely linoleic, linolenic and arachidonic acids. Most animals can, however, synthesise arachidonic from linoleic acid, thereby reducing the requirement for this acid. At first relegated to a relatively minor structural role in lipids of the cell membrane, arachidonic acid and its homologues have now been shown to be the primary precursors of a whole cascade of the most potent biological substances yet discovered. These eicosanoids may be regarded as hormones with largely local effects on smooth muscle, platelet aggregation, inflammatory response, neurotransmission and many other physiological systems. Furthermore, these hormones are unusual in that they are not stored in any form but are rapidly produced on demand.

In order to better understand the mechanism of action of these compounds, it is necessary to examine the pathways responsible for their synthesis and their breakdown.

ARACHIDONIC ACID

Desaturation and elongation of the 16 carbon fatty acid chain produced by the extramitochondrial lipogenic pathway is accomplished by enzymes associated with microsomal particles in the cytoplasm. The enzyme, fatty acyl-CoA delta-9 desaturase complex that is responsible for the introduction of a double bond (*cis* configuration) between carbons 9 and 10 of the fatty acid chain is fairly ubiquitous in its distribution. Further double bonds (all of the *cis* form) may be inserted either between this first double bond and the carboxyl group (as is the case in animals) or distally (only in plants). One of the latter unsaturated acids, linoleic acid, is the precursor of the synthesis of arachidonic acid and is therefore regarded as an essential component of the diet. In parallel fashion, the trienoic fatty acid, linolenic acid, leads to the formation of an homologue of arachidonic acid known as eicosapentaenoic acid[6] (Table 1 and Fig. 1). Both of these eicosenoic acids serve as precursors for the family of hormones known as the eicosanoids which embrace the prostaglandins, prostacyclins, thromboxanes and leukotrienes.

Table 1: **Fatty acid nomenclature**

No. of C atoms: == bonds	Common name	Systematic name
18:0	Stearic	n-Octadecanoic
18:1	Oleic	*cis* 9-Octadecenoic
18:2	Linoleic	*cis* 9,12-Octadecadienoic
18:3	*alpha*-Linolenic	*cis* 9,12,15-Octadecatrienoic
18:3	dihomo *gamma*-Linolenic	*cis* 8,11,14-Octadecatrienoic
20:0	Arachidic	n-Eicosanoic
20:4	Arachidonic	*cis* 5,8,11,14-Eicosatetraenoic
20:5	EPE, Timnodonic	*cis* 5,8,11,14,17-Eicosapentaenoic

Despite the wide distribution of polyunsaturated long-chain fatty acids in the body, they form a very small part of the intracellular fatty acid pool and are stored covalently bound to the phospholipid (chiefly lecithin) fraction of the cell membrane[15]. These phospholipids consist of a 3-phospho-glycerol entity with a saturated acyl group attached at the C-1 position, the eicosenoic acid at position C-2 and a phosphate group at position C-3 linked to an amine such as choline, ethanolamine or serine.

TRAUMA

As long as the cell remains intact (no hypoxia, local inflammation or other source of chemical or mechanical damage) these phospholipids fulfil a largely structural role. However, damage to the cell membrane not only exposes the constituent phospholipids to enzymatic attack, but also releases and activates phospholipase A2, an enzyme that specifically catalyses the hydrolysis of the ester bond in position 2 of the phospholipid to release the polyunsaturated fatty acid attached to that site[8]. The released eicosenoic acids then provide the impetus for the initiation of the eicosanoid cascade that follows, as shown in Fig. 2.

PATHWAYS

Arachidonic acid (as well as other polyunsaturated acids) serves as precursor for 2 pathways, one initiated by at least 2 enzymes, 5-lipoxygenase and 12-lipoxygenase, leading to the leukotriene group of non-cyclic eicosanoids[26] and the other by a cyclo-oxygenase/peroxidase combination responsible for the cyclic eicosanoids, the thromboxanes (including the various hydroxy-eicosatetraenoic acids, chiefly 5-HETE and 5,12 di-HETE), the prostaglandins and the prostacyclins[25]. The relative activities of these primary enzymes may play a major role in controlling the physiological balance between the cyclic and non-cyclic eicosanoid hormones. While cyclo-oxygenase is present in all cells of the body

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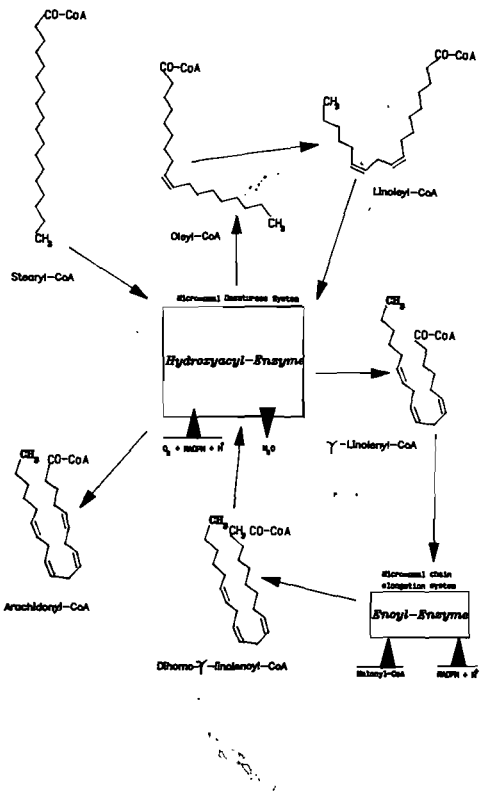

Fig. 1: Desaturation and elongation of stearyl-CoA

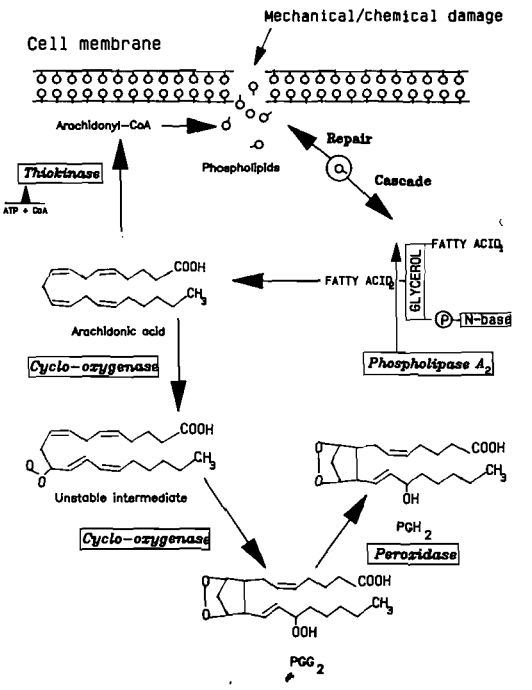

Fig. 2: Initiation of prostenoid cascade by mechanical or chemical damage, leading to the formation of arachidonic acid. Also shown are the primary reactions common to the prostaglandin, prostacyclin and thromboxane pathways.

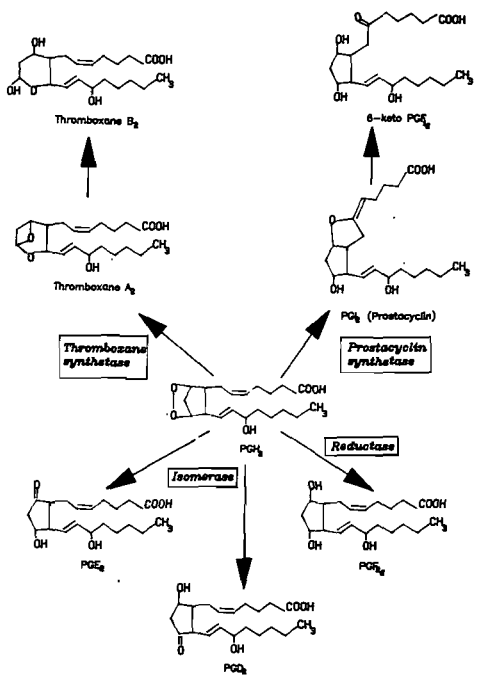

Fig. 3: Pathways leading to the formation of prostacyclin, the prostaglandins and the thromboxanes.

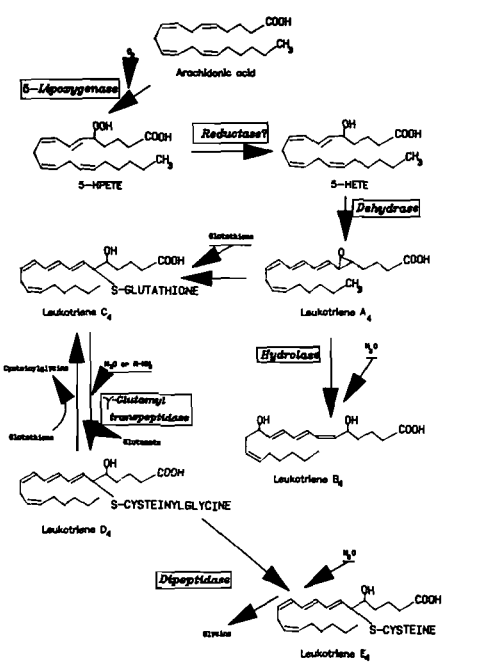

Fig. 4: Formation of the various leukotrienes from arachidonic acid

except mature erythrocytes, 5-lipoxygenase has been found in lung tissue, platelets and leukocytes (neutrophils, eosinophils, monocytes/macrophages, basophils and certain mast cell types)[4 17].

Prostanoid and thromboxane pathway

The cyclo-oxygenase enzyme system converts arachidonic acid to the cyclic endoperoxide PGG_2, which contains a 5-membered pentane ring. This, in turn, is converted by another enzyme, a peroxidase, to the more stable hydroxy form, PGH_2 (see Fig. 2), from which all other series 2 prostanoid and thromboxane hormones are derived[25]. These 2 precursors are very unstable and have a biological half-life of 3 to 4 min[23].

Prostaglandins

Two of the best known prostaglandins, PGE_2 and PGF_2, are formed from PGH_2 by the action of an isomerase and a reductase enzyme respectively[24]. The E group was named for its solubility in ether, while the F group is soluble in a phosphate buffer. As can be seen from Fig. 3, the series 2 prostaglandins all contain 2 double bonds. While these appear to be the most ubiquitous of the prostaglandins, others containing one or 3 double bonds (series 1 and 3 respectively) have also been isolated and are derived from dihomo gamma-linoleic acid and timnodonic acid respectively via a series of reactions that are similar to those of series 2[25].

Prostacyclins

Prostacyclin synthetase causes a rearrangement of the endoperoxide ring to form a cyclic oxide structure adjacent to the original ring to give an unstable prostacyclin PGI_2, which is rapidly degraded (half-life = 4 min) to the relatively stable but inert 6-keto-$PGF_{1\alpha}$[19], as shown in Fig. 3.

Thromboxanes

Thromboxane synthetase acts on PGH_2 to form TXA_2, which is unstable and has a very short half-life (30s). TXA_2 contains a 6-membered oxane ring and is rapidly broken down to TXB_2, which is relatively stable, but, like 6-keto-$PGF_{1\alpha}$, biologically inert[10]. These are summarised in Fig. 3.

Leukotriene pathway

The 5-lipoxygenase enzyme, tightly regulated by several factors including calcium[16], catalyses the addition of molecular oxygen to C-5 of arachidonic acid to give 5-hydroperoxy-eicosatetraenoic acid (5-HPETE)[5]. An unstable epoxide intermediate which forms from 5-HPETE may be hydrolysed by water to form another closely related active molecule 5,12 dihydroxy-eicostetraenoic acid (5,12-diHETE), or may react with a peroxidase enzyme to give the more stable form known as 5-hydroxy-eicostetraenoic acid (5-HETE), the precursor of all the other leukotrienes[3]. There still appears to be some controversy, however, as to whether the direct pathway proceeds via 5-HETE or 5-HPETE[15].

A hydrogen is lost from C-10 of 5-HETE, resulting in the oxygen of the hydroxy group forming a cyclic bond between C-5 and C-6 to give leukotriene A_4 (LTA_4), which may either be hydrolysed to produce LTB_4 in a reaction analogous to the hydrolysis of 5-HPETE, or may be conjugated to glutathione or some portion of the molecule[3 22]. LTC_4, which contains the entire glutathione molecule, may be converted to LTD_4 by the removal of glumatic acid, via the action of a gamma-glutamyl transpeptidase which requires the presence of a free glutathione molecule[27]. LTD_4 may, in turn, be converted to LTE_4 by the futher removal of glycine by means of a simple hydrolysis[2]. Most of these leukotrienes have been identified in the complex of substances originally isolated as SRS-A, or slow-reacting-substance of anaphylaxis[15]. This pathway is summarised in Fig. 4.

Similar compounds are formed from other polyunsaturated fatty acids, where an eicosatrienoic acid leads to the synthesis of the leukotriene 3 group, while an eicosapentaenoic acid gives rise to the leukotriene 5 group. Furthermore, the peroxy group has also been found in at least 3 other positions, for example 8,9-leukotriene and 11,12-leukotriene[11 12 13].

CATABOLISM

Although all prostaglandins, except PGI_2, are rapidly removed by the lungs (more than 95% after one circulation[7]), some are also broken down locally in the tissues where they are produced. As a consequence, most prostaglandins have a half-life in the circulation of less than 5 min[21]. Prostacyclin (PGI_2), on the other hand, is synthesised in the lungs and catabolised by peripheral tissues, and can therefore cause systemic reactions[18 20].

The leukotrienes are cleared from the circulation by hepatic uptake and biliary excretion[1]. Approximately 40% and 60% of an injected dose are eliminated via the urine and faeces respectively[14].

MECHANISM

The available evidence suggest that prostaglandins act by modulating the concentrations of intracellular calcium, adenyl cyclase and therefore cAMP[18]. While the physiological action of the prostaglandins is largely vascular (e.g. prostacyclin and PGE_2 cause smooth muscle relaxation while $PGF_{2\alpha}$ and TXA_2 cause smooth muscle constriction, prostacyclin and TXA_2 respectively inhibit and stimulate platelet aggregation), they have also been implicated in the inflammatory response, transmission of neural impulses at myoneural junctions, stimulation of cell growth and many other functions[21].

Although receptors for LTB_4 have been demonstrated on monocytes[9], the internal second messenger system has not been identified.

CONCLUSION

The eicosanoid family of hormones comprises a large number of different structures, which are part of a physiological control system that is geared to react instantaneously to changes in the homeostasis of organ systems. Despite the apparent complexity and diversity of these structures, recent advances in the elucidation of the chemistry and biochemistry of these eicosanoids have led to the discovery of the initiating cascade of events, starting with the rupture of the cell membrane and ending with the production of the specific eicosanoid/s required to counter the damage on a localised basis. The thrust of current research is to determine the mode of interaction with the cell as well as the exact mechanism of the second messenger system within the cell. Realisation of this goal will not only further advance our understanding of this first-line of cellular control, but may lead to startling advances in drug development.

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INTERNATIONAL TRAINING IN ANIMAL CARE: ROYAL VETERINARY COLLEGE, LONDON

For most of its long history the Royal Veterinary College (RVC) in London has trained veterinary surgeons and sent them all over the world to pursue their profession. They care for sick animals, help control and reduce outbreaks of disease, help run programmes to improve breeding and farm animal production, and monitor food hygiene while in many instances undertaking research projects to improve veterinary medicine worldwide.

Such research often provides spin-offs for human medicine. The college is now a semi-autonomous school of the federal University of London whose objectives are to pursue excellence in teaching, research and scholarship.

From its modest beginning nearly 200 years ago in 1791, when 14 students took a two-year course, the college has expanded and adapted to meet a changing world by emphasising the modern scientific approach and employing advanced technology.

In addition to its undergraduate training for surgeons and nurses, it has two postgraduate Master of Science courses and numerous opportunities for research. It has become the largest veterinary school in the United Kingdom with over 100 academic staff, about 350 full-time home and overseas students, studying over five years for the degree of BVetMed, plus (in 1987-88) 42 full-time and 34 part-time postgraduates.

The college also has a large and continually changing nucleus of attached and visiting research workers from countries such as Iraq, Algeria, Turkey, Nigeria, Malaysia and New Zealand. Support staff include technicians, veterinary nurses, animal attendants, receptionists, secretaries and house staff.

Degree Courses

The main undergraduate course is unique. In 14 terms the intensive BVetMed course meets all the requirements of the European Community's Veterinary Directive and it is followed in all cases by a one-term elective period of further specialisation which may be taken at the college or elsewhere in the United Kingdom.

The choice of elective is being widened and in 1988-89 four students will take the Food Hygiene elective at the Royal Veterinary and Agriculture University, Copenhagen, while four Danish students will take one of the companion animal electives offered at the RVC.

The college offers two MSc-taught courses in animal health in the Department of Veterinary Medicine and Animal Husbandry, and in laboratory animal science in the Department of Veterinary Pathology. This latter was specifically designed to train graduates to meet the needs of Britain's Animal (Scientific Procedures) Act 1986, which replaced the 1876 Cruelty to Animals Act.

Uniquely it offers a choice of full or part-time study on a modular basis and is open to medical and biological graduates as well as veterinarians. Research forms an important aspect of the course.

The philosophy of the course rests on four precepts:

- Only when it is absolutely necessary, totally justified and when there is no satisfactory alternative, should animals be used in a laboratory.
- Only the minimum number of animals should be used, of the minimum size necessary and for the minimum period of time.
- Only the most humane and painless techniques and methods should be employed.
- Only those persons who always remember their ethical and moral obligations should be allowed to use laboratory animals.

Research projects may be undertaken at the RVC or another approved institution to increase the flexibility of the course structure. They have a two-fold importance: to stimulate an interest by the students in research which may continue into further study and as training for a later stage of their careers, and ensuring that the students understand the problems, pitfalls and feasibility of research with animals.

One of the problems any veterinary group faces is that the amount of money available for research is limited and often there are few sources of funding unless the work has an impact on human medicine. In the present climate another factor is the impact of research findings.

The progesterone detection method of pregnancy diagnosis in cattle, which is now standard practice, was developed and patented jointly between the RVC and the Institute of Animal Physiology at Babraham, eastern England.

The United Nations Food and Agriculture Organisation (FAO) is currently financing a number of projects run in conjunction with the Zambian Department of Tsetse Control Services (DVTCS).

Joint Research

One of the two major projects undertaken by the RVC African research team formed part of a long-term study under an FAO-financed acaricides and production programme, led by Dr Rupert Pegram which investigated various aspects of the economics of tick control. The team researched the impact of intensive tick control and feed supplementation on the milk production of indigenous cattle.

The second project also run with FAO and DVTCS help involved ascertaining the prophylactic period of Samorin in the study of trypanosomiasis. The next student-organised research team study is expected to be in 1990.

Internationally the college is involved in joint research projects with institutions in the United States and developing countries, the latter being supported largely by Britain's Overseas Development Administration. Within the European Community (EC) there are research studies supported by EC funds with veterinary colleges in Copenhagen, Utrecht and Munich.

As it approaches its third century the college is eagerly perparing for an exciting and challenging future at the forefront of veterinary research and teaching.

Polly Curds, Royal Veterinary College, London, UK.

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