ISSN 0038-2809

Journal of the South African Veterinary Association

Tydskrif van die Suid-Afrikaanse Veterinêre Vereniging

e/Junie 1989

Volume • Jaargang 60 No. 2

Wurm-weerstand in Sybokke

Journal of the South African Veterinary Association
Tydskrif van die Suid-Afrikaanse Veterinêre Vereniging

June/Junie 1989 **Volume • Jaargang 60 No. 2**

SA ISSN 0038-2809
Dewey Cat No. 636 089

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Front page/Voorblad:
Angora goat sale

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Printed by/Gedruk deur:
Beria Printers/Drukkery,
Pretoria

Contents • Inhoud

Geldelike steun deur die Departement Nasionale Opvoeding word met dank erken.
Financial subvention by the Department of National Education is gratefully acknowledged.

Intralesional triamcinolone hexacetonide in hypertrophic scarring in a horse

We wish to report the successful treatment of a progressively deforming vertical upper eyelid scar in a Thoroughbred mare after 2 intralesional injections of triamcinolone hexacetonide (Lederspan, Lederle Laboratories). This progressive scar growth and contracture followed a superficial laceration of the upper eyelid, sustained during emergency surgery. Inability to close the eyelid and threatening corneal abrasion required urgent measures.

An initial dose of 0,5mg triamcinolone hexacetonide was administered by one of us (S.P.), the owner, through a tuberculin syringe and size 24 needle, in the 25mm long scar with minimal initial improvement. This was followed 3 weeks later with a 1mg dose whereafter involution of the hypertrophic, contracted scar occurred rapidly over a period of 2 weeks. The eyelid is now completely mobile and supple. Triamcinolone hexacetonide is a synthetic fluoroglucocorticoid ester that is obtained by the chemical addition of an acetonide group over the 16 and 17 hydroxyl positions and a butylacetate radical at position 21 of triamcinolone. The rate of release of triamcinolone from this hexacetonide ester is delayed and therefore a prolonged effect on tissue at the local injection site is to be expected. It is frequently used in the treatment of keloids and hypertrophic scars in humans, especially since no systemic side-effects are known to occur when used intralesionally. As far as we could ascertain, this drug has not been previously used in the treatment of hypertrophic scarring, an unusual occurrence, in horses.

Increased collagen synthesis in hypertrophic scars (in humans), as measured by proline hydroxylase activity, has been described[1]. Intralesional triamcinolone does not inhibit this rate of collagen synthesis[3]. Eisen[5] discovered that certain alpha globulins (alpha -1 antitrypsin and alpha-2 macroglobulin) inhibit collagenase activity. These collagenase inhibitors were found in increased amounts in keloids and hypertrophic scars[2]. Current data suggests that triamcinolone removes these alpha-globulin collagenase inhibitors, thereby allowing collagenase to break down the excessive hypertrophic scar collagen[4].

We sincerely hope that this short case report will stimulate further research in the use of triamcinolone hexacetonide in severe hypertrophic scars in horses, instead of, or in addition to surgery.

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USE OF ANABOLICS IN BEEF PRODUCTION*

DEFINING THE PROBLEM

By the middle of 1982 the world population was estimated to be 4 000 - 5 000 million people of which 72% lived in developing countries, China included. By the year 2 000, an increase to 6 000 to 8 000 million is envisaged, with the disquieting qualification that the fastest growth rate is expected in the developing world (2-5% p.a. as against 0,9% in the developed countries). By contrast, the total food production in the whole world can barely keep pace with the population growth, resulting in stagnation of food production per population unit. Food production implies protein production. On a world basis, vegetable protein is responsible for 70% of the proteins in human nutrition, whereas animal protein supplies only 30%.

Presently the source for this demand consists of approximately 9 000 million animals, comprising 3 000 million head of cattle, 600 million pigs and 5 400 million units of poultry. Dramatically, 70% of this source is present in the developing countries, which supply only 34% of the total world meat production and 20% of world milk production. This clearly emphasises the threatening discrepancy between production of and demand for animal protein. It should also be borne in mind that for this production of animal protein, mainly vegetable proteins are required.

The total annual protein requirement for the whole animal production world-wide is estimated at more than 500 million tonnes. By contrast, animal production only contributes 50 million tonnes of animal protein for human consumption. This implies a ratio of about 10 to 1 for the conversion of vegetable protein to animal protein. Ruminants alone already consume more than 300 million tonnes of this total figure of proteins fed to production animals. A large proportion of plant protein is derived from grains and/or oil-containing seeds. In the developed countries, this proportion even exceeds 90%, which means that less than 10% of grains is being processed for human consumption and that animal production to a large extent competes with the needs of human nutrition. In addition, the price of many of these protein-rich vegetable feeds has increased markedly in recent years. The increasing need for animal feed concentrates, together with this price increase, threaten to inhibit the expansion of animal production even in the developed countries. In view of this challenge there has been an active search over the last decades for so-called "growth factors" to enhance the efficiency of animal production.

During the past few decades, technical and economic imperatives in the developed countries have effected a significant modification in the production of animal proteins. This modification has been enforced on the one hand by economic prosperity with, in its wake, the increasingly critical demand by the average prosperous person concerning quantity and quality of his food package and on the other hand the ever-growing interest and participation of industry in production activities, the so-called vertical integration. Under the influence of both these factors, the smaller family concerns became replaced more and more by large industrial production units, supervised or managed by a few specific industries. As in such industries everything is geared to the highest possible return at the lowest possible cost, it is readily understood that all possible means were sought to enhance the returns and to minimise the health hazards, which are greater in such concerns. Such means were gratefully offered by the simultaneous evolution in the pharmaceutic industry. The latter was quick to realise the potential of such a market, consequently intense research was instituted to continually develop new remedies that could influence animal production. This has led to the use of a variety of "growth factors", to be added to feeds or administered directly to the animals. In beef production, use is made of two groups of growth stimulants according to the pharmacotherapeutic properties of each: antibiotics which are legally permissible in certain feeds under specific restrictions and hormonal substances used in calf fattening as well as for adult cattle, albeit illegally in most European countries.

USE OF ANABOLIC HORMONES AS GROWTH STIMULANTS IN ANIMAL PRODUCTION

1. What are anabolic hormones?

   Anabolic denotes the characteristic of stimulating the building of tissues, more precisely, building up of proteins in the body. This protein increase occurs mainly in the muscular tissues, so that an anabolic effect factually means greater muscle accretion. As nitrogen forms the main basic ingredient for such protein build-up, greater nitrogen consumption occurs, and less nitrogen is excreted in the urine than is ingested in the food, in other words a positive nitrogen balance is attained. Substances which stimulate this mechanism are termed anabolics. Certain sex hormones have anabolic activity besides stimulating sexual activity.

   In animal production, and particularly in the case of cattle, the anabolics comprise not only all the sex hormones of steroid nature, i.e. oestrogens, androgens and gestagens, but also some non-steroids having oestrogenic activity. Thus in the process of meat production, preparations are used with exclusively sexual activity, and then mainly with oestrogenic action, which is totally different from anabolic therapy in man. In fact, in cattle the oestrogens or female sex hormones are more specifically anabolic than the androgens or male sex hormones.

2. Classification of the anabolic hormones.
   Besides a classification on the basis of their hormonal activity, the anabolic hormones are also subdivided into natural (endogenously produced) and synthetic sex hormones, exogenous by nature. As the naturally occuring substances can also be prepared synthetically, and because toxicity and activity are mainly determined on the basis of their occurrence naturally in the body (endogenous) or whether they are introduced foreign substances (exogenous), there is an increasing tendency to distinguish between endogenous ("liggaamseie") and exogenous ("liggaamsvreemde") preparations. The non-steroid preparations represent an entirely separate, exeptional category, solely for the oestrogens. An overview of the anabolic hormones most commonly used in beef production is given in Table 1.

ANABOLIC EFFECT

For the sake of economic viability, the conversion of foodstuffs to meat of high nutritional quality must take place as efficiently as possible. This is the role demanded from an anabolic substance. In the evaluation of anabolics in this role, 3 aspects must be borne in mind: enhancement of growth, food conversion and carcass quality. The ratio of food intake to mass increment is improved by approximately 5-10% in the case of oestrogens, which roughly corresponds to 50% in growth improvement.

It is often alleged that the quality of the meat is inferior and that the carcass contains more water. This is incorrect. There is an increased N-retention and thus protein increment. Fat deposition is decreased. As muscle tissue contains more water per unit mass than fat, the carcass does in fact contain more water in absolute measure, but percentage-wise, less.

MECHANISM OF ACTION

As a result of numerous studies, it has definitely been proved that N-retention in animals treated with anabolics increases markedly. This is not the result of increased N-resorption, but rather of decreased N-excretion. In the conversion of food protein to meat protein, 3 fractions can be distinguished: an undigested and thus unabsorbable fraction excreted with the faeces, which percentage-wise remains unchanged during the whole growth period (5%); a digested and absorbed fraction retained in the body and converted to tissue proteins via the intermediary metabolism, to both edible (meat) and inedible (hair, skin, viscera and bone) proteins; a digested and absorbed fraction not retained in the body, but broken down (enzymes, cell wear) to non-protein nitrogen and excreted in the urine.

* Address delivered at Pretoria on April 15th 1986 at a meeting of the Northern Transvaal Branch of the South African Veterinary Association

Table 1: **Anabolic hormones most commonly used in beef production**

	Endogenous	Exogenous	
	Steroids only	Steroids	Non-steroids
Oestrogens	Oestradiol Oestrone Oestriol All three orally inactive	Ethinyloestradiol Mestranol Both orally active	**Stilbenes:** Diethylstil-boestrol (DES) Dienoestriol Hexoestriol All three orally active **Myco-oestrogens:** Amongst others resorcylic acid lactone (RAL) or zeranol: orally less active
Gestagens	Progesterone Orally inactive	Medroxyprogesterone acetate (MPA) Melengestrol acetate (MGA) Both orally active	
Androgens	Testosterone and esters The steroid active principle is set free by hydrolysis. Orally inactive	Methyl testosterone (Orally active) 19 nortestosterone and derivatives (Orally inactive) Trenbolone (Orally inactive)	

When, during the growth process, anabolics are administered, the N-retention increases whilst the fraction excreted in the urine decreases. The percentage protein converted to body protein increases from 40 to 60%. Nevertheless, this depends on the period of administration. The maximal effect is attained after about 3 weeks, so that the administration should not occur too soon before slaughter. The maximum effect may even rise to 68%.

The biochemical mode of activity depends upon influencing the intermediary metabolism. This may take place in different ways for oestrogens and androgens. Both have a direct influence on the binding of steroid receptors in the sarcoplasm, with increased mRNA and protein synthesis as a result. In the case of oestrogens this takes place by increasing the growth hormone (GH). It has been determined that there is an increase in the mass of the hypophysis (30%). Although the concentration of growth hormone per units mass of the hypophysis remains the same, there is nevertheless a significant increase in the total amount of growth hormone in the hypophysis. This effect is maximal 3 weeks after the beginning of treatment if combined with energy-rich nutrition. This proves that there must be sufficient energy supplied to take full advantage of the anabolic activity of oestrogens in the growing young animal. Cessation of oestrogen administration causes the GH concentration to subside to normal within a week. The GH increase after 17-beta-oestradiol is maximal at a dosage level of 14-25 μg day^{-1}. Both the average daily increase in mass as well as the ratio of food consumption to mass increase, is optimal at 14μg day^{-1}. Oestrogenic activity plays a key role in anabolic stimulation. After direct administration of GH itself, the same effects are obtained as with oestrogen administration. Briefly, GH increases by approximately 20% and improves the food conversion rate by approximately 15%. It brings about the same modification in carcass composition with an increase in protein and decrease in fat content.

Table 2: **Summary of half-life of various anabolics**

	Species	**Half-life**
17 β-oestradiol	Human	20 min
Progesterone	Human	20 min
Testosterone	Human	10 min
Trenbolone acetate	Cow	2 hours
Diethylstilboestrol	Monkey	3 hours

TOXICOLOGICAL ASPECTS OF THE USE OF ANABOLIC HORMONES

In the study of their eventual toxicity, two important aspects must be considered: the residues both with respect to quantity and quality; the risks such residues entail in terms of public health.

Residues

As with any pharmacological substance, the fate of anabolic hormones in the body after administration will depend on pharmacokinetic parameters, such as absorption, protein-binding, tissue diffusion, metabolism and elimination. The sum total of these processes will determine how rapidly the anabolic is eliminated from the body, either in its original form, or as metabolite. Provided all these processes follow first order kinetics and the administration of the hormone is based on repeated administration or on the implanting of preparations, the concentration thereof will increase until a steady state is attained, where elimination and uptake are in balance. Such a steady state concentration of the anabolic and its eventual metabolites constitute the residue package that can be determined in the carcass after slaughter. On ceasing application, or cessation of absorption, an exponential decrease in the residue package is encountered. The term "elimination half-life" is associated with this decrease. This means the time period in which the concentration of the anabolic has decreased to half the orginal value. Consequently, substances with a long half-life, may cause danger of accumulation in the body and a larger residue package.

Quantitative aspects

Quantitatively the residue package will thus depend upon the previously mentioned pharmacokinetic parameters. These may be influenced by a wide range of factors, such as the age of the animal, the form in which the anabolic is administered, the injection site, the solute, the dosage and dose interval, the withdrawal period, and last but not least in any way, the nature of the preparation. Here a distinction must be drawn in the first place, between endogenous ("liggaamseie") and exogenous ("liggaamsvreemde") preparations, the latter of which usually have a longer half-life. In Table 2 the half-lives of the various anabolics are given by approximation after a single administration. The difference between half-lives is of importance for toxicological evaluation. After

Table 3: **Teratogenic effect of various sex hormones**

Species	Oestradiol	Progesterone	Testosterone	Oestradiol + Progesterone + Testosterone	DES
Monkey		—	+		
Guinea pig			+		
Dog	a		+		
Rabbit	—	+	+	—	—
Mouse	+, b, c		+		+, b, c,
Rat	+	+	+	—	+
Sheep		—	+		
Pig			+		

\+ Teratogenic effect on reproductive organs
— No effect
a Hip abnormalities
b Cleft palate
c Heart abnormalities

subcutaneous implantation of trenbolone, a half-life from the depot of a few weeks to 2 months is given. The short half-life (20 min) for DES in adult animals, and the longer period in young animals (because of the lesser microsomal liver enzyme activity), indicate that possible deleterious effects will most likely appear in juveniles.

Qualitative aspects
Qualitatively the composition of the residue packet will depend on the metabolic pattern specific for each individual anabolic. Endogenous anabolics are usually metabolised rapidly and to a high degree to inactive metabolites during their first passage through the liver. Hence their relatively slight oral activity, which is of importance when their uptake by the consumer by way of treated meat is considered. Exogenous anabolics are generally reasonably resistant to biodegradation.

Zeranol is derived from, and is chemically closely related to, zearalanone, a naturally occuring myco-oestrogen found in feeds as a result of infection of grain by Fusarium fungus. Both are beta-resorcylic acid lactones (RAL's). In all species of animals it is converted to zearalanone, and in cattle it is further converted to the 7-beta-epimere of zeranol or beta-zearalanol (taleranol). Diethylstilboestrol (DES) mainly undergoes hydroxylation.

Risks entailed in the use of these residues
All anabolics, except DES intraperitoneally, have a low acute toxicity.

Hormonal and endocrine effects: The endogenous anabolics are known to influence reproduction, hence their therapeutic use in functional disorders of the reproductive organs. Zeranol is 100-1 000 times less active hormonally than oestradiol. Trenbolone is 5 times more anabolic and 3 times more androgenic than testosterone. DES has 100 times greater affinity for the oestrogen receptors than oestradiol.
Teratogenicity: The endogenous anabolics, especially testosterone, and to a lesser degree progesterone and oestradiol, as well as the exogenous DES, at high doses are teratogenic and embryotoxic in various animal species. It has also been shown that DES is teratogenic in man. In the case of zeranol and presumably also in the case of trenbolone no indications of teratogenicity exist.
Mutagenicity: With the exception of possible mutagenicity of DES and an as yet not clearly defined cytotoxicity of trenbolone, no indications of mutagenic effects of oestradiol, progesterone, testosterone or zeranol have been established.
Carcinogenicity: According to the Internation Agency for Research on Cancer (IARC) there is sufficient evidence for carcinogenicity in experimental animals in the case of oestradiol, testosterone and DES, and limited evidence in the case of progesterone. In the case of oestrogens, there are strong indications of possible induction of endometrial carcinomata in women. By epidemiological investigation it has been shown that DES induces cervical and vaginal tumours in daughters between the ages of 10 and 30 years of whom the mothers were treated during pregnancy with doses of DES varying from 135 mg to almost 20 g per pregnancy. A transplacental embryonally induced carcinogenicity therefore exists. In the case of progesterone and testosterone no epidemiological data exist concerning possible carcinogenicity in man. Extensive studies on zeranol yielded no positive evidence for carcinogenicity of this preparation. It is of special importance to consider the mechanism whereby hormones may induce tumours. Carcinogenesis may be produced by a genotoxic mechanism, thus with mutagenic effects, or by epigenetic mechanisms whereby the genetic code is not altered in any way. Although numerous carcinogens are mutagenic, there are no indications, for the anabolics with the possible exception of DES. Nevertheless, there are indications that DES, and more particularly its metabolites, are bound covalently to DNA. There are strong indications that the other anabolic hormones function indirectly, in as much as they promote the development of tumours, thus an epigenetic effect, associated with their hormonal activity. With the exception of DES, which eventually may be shown to act genotoxically, one may assume that for the other hormones, in view of their epigenetic effect, a threshold value or "no effect level" exists, in contrast to the true carcinogens,

Table 4: **Oestrogen content in various foods compared to meat from oxen treated with oestradiol**

Treated meat	33 pg g^{-1}
Meat from pregnant animal	2 500 - 5 500 pg g^{-1}
Milk from non-pregnant cow	80 pg ml^{-1}
Milk from pregnant cow	126 pg ml^{-1}

Table 5: **Progesterone content in various foods compared to meat from oxen treated with progesterone**

Treated meat	0,5 ng g^{-1} (19,5 ng per 157 g meat, 25% fat = 39g)
Full cream milk	9,5 ng ml^{-1}
Cream	73,0 ng ml^{-1}
Butter	133 ng ml^{-1}

LEGAL REGULATIONS CONCERNING THE USE OF ANABOLICS
The guide-lines laid down by the European Council on July 31 1981 concerning the prohibition of certain substances with hormonal and thyrostatic activity, provide

Table 6. **Relative consumption of oestrogens per day**

Age	Food packet	Oestrogen in milk / Oestrogen in meat
Child under 1 year	696 g milk and derivatives 7 g meat	$\frac{.696 \times 100}{7 \times 22} = \frac{450}{1}$
child 6-8 years	538 g milk and derivatives 38 g meat	$\frac{538 \times 100}{38 \times 22} = \frac{13}{1}$
Adult	318 g milk and derivatives 110 g meat	$\frac{318 \times 100}{110 \times 22} = \frac{13}{1}$

Table 7: **Relative consumption of progesterone per day**

Age	Progesterone in milk / Progesterone in meat
Child under 1 year	$\frac{7\,300}{1}$
Child 6-8 years	$\frac{1\,000}{1}$
Adult	$\frac{200}{1}$

the frame-work for handling the problem concerning the use of anabolics in all the EEC member states. They are the following:

- Prohibition of the administration to farm animals of thyrostatics, oestrogens, androgens and gestagens; the sale for slaughter of animals so treated; the sale of such meat; the processing of such meat.
- Prohibition of the sale of stilbenes and thyrostatics
- Use of hormones, other than the stilbenes are allowed for therapeutic purposes.
- Article 5 charges the Council to come to a unanimous resolution as soon as possible concerning the eventual permission for the use of 17-beta-oestradiol, progesterone, testosterone, trenbolone and zeranol for finishing purposes. In the interim the national prescriptions remain valid, and no new substances are permitted to be used. Meanwhile the Council had decided to halt all activities already in progress concerning advice in respect of Article 5 (Working Group on Anabolics). As from January 1 1988 all hormones used in beef production, have been forbidden, also even in those countries where certain substances were still permitted.

  The issuing of this prohibition most probably will not be followed up by enforcement, so that the existing illegality will not be changed. Instead of protecting public health, it will have the opposite effect. Those who make use of these hormones, even if they desist from the use of the feared stilbenes and are conservative towards the other exogenous

Table 8: **Daily production of endogenous hormones compared to the endogenous intake by way of meat from treated animals: ratio of oral intake of treated to daily endogenous hormone production**

		Oestrogens	Progesterone	Testosterone
Woman		$\frac{1}{13\,000}$ (Menopause) to $\frac{1}{18 \times 10^6}$ (pregnancy at term)	$\frac{1}{16\,900}$ (Menopause) to $\frac{1}{15 \times 10^6}$ (Pregnancy at term)	$\frac{1}{8\,800}$ (Menopause) to $\frac{1}{20\,000}$ (puberty)
Man		$\frac{1}{39\,700}$	$\frac{1}{21\,000}$	$\frac{1}{400\,000}$
Child (prepubertal)	Female	$\frac{1}{15\,700}$	$\frac{1}{20\,000}$	$\frac{1}{2\,000}$
	Male	$\frac{1}{12\,100}$	$\frac{1}{12\,200}$	$\frac{1}{4\,000}$

* Daily oestrogen production:
Pregnant woman: 4 000 to 64 000 ug
Child: 42 to 54 ug

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substances, know only too well that in the use of endogenous hormones, control is ineffectual. Even if they are detectable and identifiable, not a single specialist can come to a final quantitative evaluation which will allow a decision to be made whether the truly detected endogenous hormones are of endogenous origin or whether they have been administered exogenously. As long as this situation persists the practice will flourish, especially by using injections, a technique which raises serious issues about the safety of the consumer. Hence a solution for the future must be sought.

PROPOSAL FOR A SOLUTION

With regard to the foregoing, a choice between two alternatives can be made: either one accepts the above guide-lines, knowing full well that they will not be followed and that the control techniques to enforce them will fail. One must then accept the situation hypocritically, fully aware that the so-called protection afforded to public health, on which the ban is based, will continually remain in the balance and one should realise that, due to the lack of certain registered implantation preparations, injectable solutions will be used with the consequent risks to the consumer. Only in the case of exogenous preparations may one eventually expect a decrease in their use, because they are identifiable and their presence indicates exogenous administration, provided of course efficient control is exercised.

One may however decline the hypocrisy and opt for the legitimate use of endogenous hormones under well-defined modalities, laid down by resolution, whereby one may offer the consumer a more guaranteed protection. Hence a proposal for solution may be made, consisting of two modes, each of which is inseparably bound to the other: regularised permission, coupled to efficient control.

REGULARISED PERMISSION

Only those products should be allowed which comply with the following 3 main conditions, in ascending order of importance:

Economic viability. On this score there can be no doubt, both for the exogenous as well as for the endogenous preparations, on conditions that the species, the time of administration and the hormonal status is kept in mind.

Carcass or meat quality may not be impaired. This poses no problem, as all investigations indicate an improvement.

No residual toxicity for the consumer may be present. In any dicision on residual toxicity the viewpoint remains valid that public health is given first priority, and that economic interests remain subordinate and secondary. When these 2 issues can be reconciled, then such as modus operandi should be given a chance, rather than promulgating illogical and impractical regulations which only perpetruate the illegality and the risks for the consumer. If this point of view is adopted, then a distinction should be drawn between endogenous and exogenous preparations: only the endogenous preparations oestradiol, testosterone and progesterone come up for consideration.

Studies on meat obtained from cattle treated under controlled conditions, i.e. in the form of an implant of endogenous hormone 60 days before slaughter, have shown that there are many reasons for a complete guarantee of absence of residues toxic to the consumer.

1. The concentrations found in meat from treated animals were lower than those that could be present in other edible products of animal origin, and also could be lower than in meat from untreated animals (see Tables 4 and 5).

2. The intake of these quantities of residues in treated meat prove to be markedly lower than via milk products (Tables 6 and 9).

3. The daily production of endogenous hormones, even in the most vulnerable age groups, namely women at menopause and prepubertal children, is very much higher than the quantity consumed by way of meat from treated animals. (Table 8).

4. Endogenous hormones exert virtually no activity when taken per os, because at most, 10% escape breakdown in the liver after absorption from the intestinal tract. Thus the abovementioned ratios may be increased by a factor of 10. For the same reason there is no danger of a cumulative effect. The fraction that escapes degradation and enters the circulation is insignificant compared to the normal endogenous production.

5. In the process of break-down, metabolites occur which are equally endogenous, and they follow the same pathway in the intermediary metabolism as do the endogenously produced hormones. Therefore they share the same affinity for receptors in the target organs, the same degree of protein-binding as well as eventual toxicity. On the basis of these reassuring facts, the endogenous hormones may be considered for the enhancement of beef production, provided certain strictly monitored modalities are complied with, such as the form (implantation), the site (ear base), qualification of the applicator (veterinarian), with a minimal withdrawal period (e.g. 60 days before slaughter) and with the possibility of implant removal within a few hours before slaughter.

Nearly all exogenous hormones, with the exception of zeranol and trenbolone, possess a strong oral activity because more than 90% escape the first liver passage break-down and enter the general circulation. Some of them have an entero-hepatic circulation, whereby the danger of a longer period of activity and even of cumulative effects arises. They have a greater affinity for the receptors in the target organs and tend to bind irreversibly to them, as result of which serious disturbances of hormonal functions may arise. The resulting metabolites are sometimes bound very strongly to proteins. Being foreign to the body for the greater part, they may interfere with the normal intermediary metabolism and cell function. In the case of some substances, such as DES, there are indications that after passage through the body of treated animals, metabolites may arise with a higher degree of activity than the original product. In addition, in the case of DES, it has been shown with absolute certainty that it is not only potentially carcinogenic for experimental animals, but that it can cause transplacental abnormal effects in the female human foetus, which in turn may give rise to a rare type of cancer of the reproductive tract during subsequent puberty.

B. AN EFFICIENT CONTROL METHOD

Permission to use endogenous substances can only be granted when at the same time tight control measures ensure that only permitted substances are used. Such control can be exercised exclusively by means of qualitative and quantitative chemical analyses. Employment of such analyses on urine from live animals during the finishing period can offer sufficient guarantees against the illegal use of prohibited exogenous substances or can detect the irregular use of permitted endogenous substances, and that for the following reasons:

1. urine concentrations are usually higher than those in meat;
2. the material is more homogenous and is obtainable in sufficient quantities to repeat the procedure if required;
3. it is of great practical importance that one has sufficient time at one's disposal: the animals can be kept alive until the results of the analyses are available. Sampling can be timed to take place 3-4 weeks prior to the end of the finishing period, which is exactly the period when hormonal application is in full swing;
4. sampling can be done on a large scale simultaneously on the same property, which is of practical convenience for large industrial feedlot concerns.

ACKNOWLEDGEMENT

Prof H.P.A. de Boom's translation of this Flemish address is sincerely appreciated.

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BENSIMIDASOOL-BESTANDE *Ostertagia circumcincta* IN SYBOKKE

P C VAN SCHALKWYK* en J SCHRÖDER**

ABSTRACT

Angora goat kids from the eastern Cape Province with natural infestations of *Ostertagia circumcincta, Nematodirus spathiger,* and *Trichostrongylus rugatus* were treated with albendazole, fenbendazole or morantel citrate, while a fourth group remained an untreated control group. The benzimidazoles were ≤ 50% effective against *O. circumcincta,* and approximately 50% effective against possibly resistant fourth larval stage *N. spathiger.*

To confirm the diagnosis of anthelmintic resistance in the *O. circumcincta,* infective larvae (L_3) were cultured from the faeces of the kids prior to treatment, and passaged through a donor sheep. Thereafter 2 groups each of sheep and goats were infested artificially with these parasites, and one group of each animal species was drenched with albendazole at 4,75 mg kg^{-1} in a second trial. The remaining groups were kept as untreated controls. This treatment was < 5% effective in both sheep and goats, confirming the resistance of this isolate of *O. circumcincta* to benzimidazoles in both host species.

Key words: Benzimidazole-resistance, *Ostertagia circumcincta,* Angora goats

Van Schalkwyk P.C.; Schröder J. Benzimidazole-resistant *Ostertagia circumcincta* from Angora goats. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 76-78 (Afr) P.O. Box 1621 Birchleigh, Republic of South Africa.**

INLEIDING

Wurmparasiete by sybokke is een van die belangrikste probleme van die bedryf[2]. Hoewel daar voorheen geglo is dat bokke net so vatbaar vir wurms is as skape, word dit nou al hoe duideliker dat sybokke inderwaarheid meer vatbaar is as skape, omdat hulle nie dieselfde mate van weerstand teen wurms opbou nie[8]. Selfs in die volwasse stadium bly bokke steeds baie vatbaar vir wurms[7 10].

Tradisioneel is aanvaar dat wurmmiddels by skape en bokke ewe doeltreffend is, maar belangrike verskille in die metabolisme van wurmmiddels kan veroorsaak dat sommige daarvan minder doeltreffend in bokke is[7].

Vermoedelike wurmmiddelondoeltreffendheid in sybokke is opgevolg om die sensitiwiteit van die wurmstam in skape en sybokke teen verskillende middels te toets.

PROEFBESKRYWING

Proef 1

Sybokke wat aangehou is op lusernweidings in die Paterson-omgewing in die Oos-Kaap is herhaaldelik met albendasool (ABZ) en fenbendasool (FBZ) gedoseer. Twintig 8 - 10 maande-oue boklammers wat tydens die voorafgaande 6 weke glad nie gedoseer is nie, is aangekoop en na die laboratorium vervoer. *Ostertagia circumcincta*-larwes is uit hierdie bokkies se mis gekweek en gebruik om 'n skenkerskaap te besmet sodat die wurmstam later verder getoets kon word.

'n Wurmeiertelling is op elke bokkie gedoen en hulle is in rangvolgorde gerangskik en verdeel in 5 herhalings van 4 bokkies elk. Die 4 diere in elke herhaling is ewekansig toegewys aan die 4 behandelingsgroepe: onbehandelde kontrole, ABZ 3,8 mg kg^{-1}, FBZ 5 mg kg^{-1}, en morantelsitraat (MOR) 14,5 mg kg^{-1}. Die behandelings is per os toegedien.

Die bokkies is 10 tot 14 d later geslag en die rondewurms in hul ingewande herwin en getel.

Proef 2

Twaalf 6-12 maande-oue syboklammers en 12 Döhne Merinolammers van soortgelyke ouderdom is aangekoop 3 weke voor die proef 'n aanvang sou neem. Elke lam is behandel met 4,8 mg ivermektien (Ivomec: MSD; dosis 209 — 1 067 $\mu g\ kg^{-1}$) 11 d voordat die kunsmatige besmettings toegedien is.

Die lammers is elkeen besmet met 3 000 besmetlike derde-stadium larwes van die gekweekte *O. circumcincta* oor 'n tydperk van 3 d. Die larwes is toegedien op filtreerpapierskyfies in gelatienkapsules.

Die proefdiere is geweeg en skape en bokke is afsonderlik in rangvolgorde gerangskik en verdeel in 6 herhalings van 2 diere elk op die dag van behandeling. Die 2 diere in elke herhaling is ewekansig toegewys aan die behandelingsgroepe: onbehandelde kontrole en albendasool teen 4,75 mg kg^{-1}. Die diere is per os behandel toe die besmetting 25-27 d oud was.

Die diere is 6-8 d later geslag en slegs die rondewurms in hul abomasums is herwin en getel.

Laboratoriumtoestande

Die proewe is uitgevoer by die Veemiddeltoetseenheid van die Suid-Afrikaanse Buro vir Standaarde in Oos-Londen. By die laboratorium is die diere in elke proef aangehou op hortjiesvloere om ongewenste wurmbesmettings te beperk.

Na slagting is die wurms soos volg herwin: In die eerste proef is die abomasum- en dunderm-inhoud deur 'n sif (openinggrootte 63 m) gewas en die inhoud van die sif vir wurmondersoek behou.

In die tweede proef is slegs die abomasumwurms herwin. Die abomasuminhoud is in 'n waterbad verwerk volgens die gewysigde Baermannmetode[12]. Alle abomasumwande is vir 3 h in 'n waterbad met 3% HCl geplaas vir vertering van die slymvlies. Die verterings is daarna op 'n sif met openinggrootte 38 μm gekonsentreer.

Wurms is makroskopies getel en verwyder uit die ingewandsinhoud van Proef 1 en die Baermannresidu uit Proef 2. Hierna is mikroskopiese tellings gedoen op 10% van die oorblyfsels. Totale mikroskopiese tellings is gedoen op die abomasumverterings en die Baermannfiltrate uit Proef 2.

Statistiese ontleding.

Die verskille in totale wurmladings en in die persentasie L_4 van onbehandelde skaap- en syboklammers in Proef 2 is ontleed met behulp van 'n Mann-Whitneytoets[13].

UITSLAG

Proef 1

Die wurmtellings en persentasie doeltreffendheid van elke behandeling word opgesom in Tabel 1.

O. circumcincta, Nematodirus spathiger, en *Trichostrongylus rugatus* is uit al die onbehandelde kontrolebokkies herwin. Een dier uit hierdie groep het ook 2 volwassenes elk van die wurmsoorte *Haemonchus contortus* en *Trichostrongylus axei* geherberg.

Die bensimidasole was <42% doeltreffend teen die volwasse *O.circumcincta* teenoor die 88% van MOR, en ondoeltreffend teen die vierde larfstadium teenoor 62% van MOR. Teen volwasse *N. spathiger* was die bensimidasole omtrent 99% doeltreffend, maar ≤54% teen die vierde larfstadium. MOR was 100% doeltreffend teen vierde-stadium larwes en volwassenes van hierdie wurm. Al 3 die behandelings was >90% doeltreffend teen vierde larfstadium en volwasse *T.rugatus.*

Proef 2

Die wurmtellings en doeltreffendheid van die behandeling word opgesom in Tabel 2.

Daar is betekenisvol meer wurms herwin uit die bokkies as uit die skaaplammers ($p < 0,05$). ABZ was <4% doeltreffend teen hierdie isolaat van *O.cicumcincta* in skape en bokke. Ongeveer 20% van die wurms wat uit die skaaplammers herwin is was in die vierde larfstadium, terwyl die verhouding by die bokkies ongeveer 8% was. Hierdie verskil was ook beduidend ($p < 0,10$).

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Ontvang: Mei 1988 Aanvaar: November 1988

Tabel 1: **Gemiddelde wurmtellings en persentasie doeltreffendheid van behandeling met albendasool, fenbendasool, en morantelsitraat by natuurlik-besmette sybokke**

Wurmsoort	ABZ + $\bar{x}$ (± S.A.)	Effek (%)	FBZ++ $\bar{x}$ (± S.A.)	Effek (%)	MOR+++ $\bar{x}$ (± S.A.) (± S.A.)	Effek (%)	Kontrole $\bar{x}$ (± S.A.) (± S.A.)
O.circumcincta							
L4	1 987 (±1 892,8)	0	2 002 (±1 135,3)	0	448 (±460,6)	62,0	1 175 (±1 221, 5)
Volwasse	2 468 (±833,4)	32,4	2 144 (±1 113,1)	41,3	427 (±351,8)	88,3	3 652 (±1 516,2)
N.spathigher							
L4	114 (±157,6)	54,0	130 (±160,7)	47,6	0	100	248 (±138,8)
Volwasse	14 (±19,6)	98,3	6 (±12,0)	99,3	0	100	808 (±1 210,1)
T.rugatus							
L4	0	100	2 (±4,0)	97,3	6 (±8,0)	91,9	74 (±83,1)
Volwasse	34 (±68,0)	99,7	2 (±4,0)	>99,9	868 (±1 081,7)	93,4	13 216 (±24 443,4)

+ABZ = albendasool ++FBX = fenbendasool +++MOR = morantelsitraat

Tabel 2: **Gemiddelde** *O.circumcincta*-**tellings en persentasie doeltreffendheid van behandeling met albendasool in skape en sybokke**

Wurm-stadium	Skape Kontrole $\bar{x}$ (± S.A.)	Skape ABZ $\bar{x}$ (± S.A.)	Effek (%)	Sybokke Kontrole $\bar{x}$ (± S.A.)	Sybokke ABZ $\bar{x}$ (± S.A.)	Effek %
L4	207 (±213,2)	198 (±138,9)	4,7	109 (±44,9)	107 (±61,5)	2,0
Volwasse	799 (± 265,2)	774 (±242,3)	3,1	1 246 (±278,4)	1 212 (±629,7)	2,7
Totaal	1 006 (±151,0)	972 (±365,6)	3,4	1 355 (±306,1)	1 319 (±682,9)	2,6
% L4	21 (±22,8)	18 (±7,6)	-	8 (±2,8)	9 (±3,2)	-

BESPREKING

Bloedkonsentrasies van verskeie wurmmiddels is laer en van korter duur by bokke as by skape en dit word dikwels as rede aangevoer waarom middels by bokke faal en terstond ook wurmstamme selekteer wat weerstandbiedend is[1 4]. Gevolgtrekkings moet egter versigtig gemaak word, aangesien daar skynbaar nie 'n algemene reël geld nie. Onlangs het Gillham & Obendorf[5] gevind dat levamisool in hulle eksperimente ondoeltreffend was teen 'n gevoelige stam van *Trichostrongylus colubriformis* in bokke, maar wel ten volle doeltreffend was teen dieselfde stam in skape. Dit is moeilik om die outeurs se afleiding te aanvaar dat die vinnige metabolisme van die middel deur die bok daartoe lei dat die middel geen effek op die wurms het nie, aangesien tetramisool en levamisool deur ander navorsers wel doeltreffend gevind is teen wurms by bokke[3 7 11]. 'n Moontlike ander verklaring vir Gillham & Obendorf[5] se bevinding is dat hul *T. colubriformis*-stam reeds 'n mate van weerstand gehad het wat deur die bok se laer plasmapeile uitgewys is.

Verskille in plasmaprofiele tussen skape en bokke is reeds bevestig vir levamisool[4] en oksfendasool[1]. Ondanks die feit dat albei middels se plasmakonsentrasies aansienlik laer is by bokke, het McKenna & Watson[9] gevind dat oksfendasool ewe doeltreffend by beide diersoorte was. In dieselfde proef was MOR effens minder doeltreffend teen sommige wurms by bokke as by skape. Die *T.colubriformis* in hul proef was egter tot 'n mate weerstandbiedend en daar kan nie sondermeer aanvaar word dat dieselfde vir 'n vatbare stam sal geld nie.

Die uitslag van ons proewe werp nie veel lig op hierdie polemiek nie, maar enkele opmerkings kan gemaak word. Die *O.circumcincta* was uiters bestand teen bensimidasole ongeag die soort gasheer, tot so 'n mate dat graadverskille tussen skape en bokke moontlik onsigbaar sou wees. Die doeltreffendheid van MOR by sybokke was egter in ooreenstemming met die geregistreerde aansprake vir skape (Banminth II: Pfizer, Reg. nr. G194, Wet 36/1947) teen al 3 wurmsoorte in die natuurlik-besmette sybokke van Proef 1.

Die doeltreffendheid van beide bensimidasole teen vierde-stadium *N. spathiger.* was aansienlik laer as wat vroeër beskryf is[14] en as wat verwag is volgens die geregistreerde aansprake (Valbazen: SmithKline, Reg. nr. G197 en Panacur: Hoechst, Reg. nr. G169, Wet 36/1947). Hierdie is moontlik 'n aanduiding van bensimidasoolweerstand, veral in die lig van die hoë doeltreffendheid van MOR in dieselfde proef. Die volwasse wurms was hoogs vatbaar vir albei bensimidasole, maar dit is moontlik dat die onvolwasse stadia tot 'n mate weerstandbiedend raak voordat dit in die volwasse stadium opgemerk word. Hierdie waarneming is al voorheen by *T.colubriformis* gemaak[5].

Die *O.circumcincta* in hierdie proewe was waarskynlik beter aangepas by bokke as by skape, soos afgelei kan word uit die hoër graad van hipobiose by laasgenoemde gasheer ($p < 0,10$). Die aantal wurms was ook hoër by bokke as by skape in die tweede proef ($p < 0,05$).

DANKBETUIGING

Die tegniese hulp van Mej Erica Hardman tydens die uitvoer van die proewe word met dank erken.

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DIE DOELTREFFENDHEID VAN ALFAMETRIEN-DEURWEEKTE OORPLAATJIES TEEN BEESBOSLUISE

J SCHRÖDER* en P C VAN SCHALKWYK**

ABSTRACT

The efficacy of 3 kinds of alphamethrin-impregnated ear tags was tested against natural *Amblyomma hebraeum*, *Boophilus decoloratus*, *Rhipicephalus appendiculatus*, and *Rhipicephalus evertsi evertsi* infestations of cattle. One type of ear tag was also tested in combination with a tail band of similar material. Ticks were counted macroscopically on their predilection sites.

Counts of *B.decoloratus*, *R.appendiculatus*, and *R.e.evertsi* on all trial animals diminished steadily during the first 7d after application of the devices. Counts on untreated control cattle had returned to their pre-treatment levels by Day 14 in the case of *B.decoloratus*, and by Day 21 for *R. appendiculatus*. *R. e. evertsi* did not regain their pre-treatment numbers during the trial period on the controls, but did so on one of the treated groups. This temporary drop could be interpreted as being the result of pyrethroid contamination from the hands of the investigators at the time of applying the ear tags, but did not interfere with the assessment of the effect of the tags.

None of the forms of treatment showed acceptable efficacy against *B. decoloratus*. Ear tags alone were not very effective against *R. e. evertsi*, but the numbers of these ticks on the perineum were diminished by the use of tail bands. Two kinds of ear tags showed superior efficacy against *R. appendiculatus*, and in the case of these, one ear tag appeared to be as effective as two.

The effect against *A. hebraeum* was evidently influenced by the distribution of the impregnated devices. Two types of ear tags were used alone and had no effect. The third type, used in conjunction with a tail band, reduced the numbers of this tick substantially.

Key words: Alphamethrin, ticks, cattle

Schröder J.; Van Schalkwyk P.C. The efficacy of alphamethrin-impregnated ear tags as a method to control cattle ticks. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 79-82 (Afr) 53 Ilkey Raod, Lynnwood Glen, 0081 Pretoria, Republic of South Africa**

INLEIDING

Dit is slegs in 'n klein deel van ons land moontlik om met beeste te boer sonder om die voortdurende probleem van bosluisbesmettings in die gesig te staar. Omdat die meergasheer-bosluise die geïntegreerde beheerstelsels wat doeltreffend teen enkelgasheerbosluise in Australië toegepas word hier onprakties maak, is boere in hierdie land grootliks op chemiese bosluisbeheer aangewese.

Tradisionele aanwendingsmetodes soos indompeling en bespuiting hou nadele in, soos onder andere die groot kapitale belegging in toerusting, die arbeid benodig vir die gereelde bymekaarmaak van die vee, potensiële beserings van vee en personeel, en die minder opvallende koste van verlaagde produksie vanweë verlore weityd. Alternatiewe aanwendingsmetodes sou dus aantreklik wees indien hulle prakties en ekonomies is.

Sulke alternatiewe metodes wat al beskryf is sluit in oorgietpreparate[5], voorwerpe soos oorplaatjies, oor-, horing-, en nekbande wat met bosluismiddels deurweek is[1,2], en sistemiese produkte soos laevlak voerbyvoegings, bolusse vir stadige vrystelling, of depot-inspuitings[3].

Hierdie proef is gedoen om die doeltreffendheid van alfametriendeurweekte plastiek oorplaatjies en stertbandjies teen natuurlike bosluisbesmettings op beeste te bepaal.

PROEFBESKRYWING

Proefdiere

Hereford-/Friestipe basterossies (n = 20) is op verbeterde natuurlike weidings aangehou waar hulle konstant blootgestel was aan natuurlike bosluisbesmettings. Die proef is gedoen op die plaas "Little Go" van die Suid-Afrikaanse Buro vir Standaarde (SABS) se Veemiddeltoetseenheid in Oos-Londen. Die proefdiere is in die voorafgaande 6 weke met geen bosluismiddel behandel nie. Die diere het vir die duur van die proef in dieselfde kamp geloop sodat hulle blootstelling aan herbesmetting so eenvormig moontlik sou wees.

Behandeling

Op 11 November 1983 (Dag −3) is die diere op grond van hul bosluistellings in rangvolgorde geplaas, verdeel in 4 groepe van 5 diere elk, en binne elke herhaling ewekansig toegedeel aan een van die behandelingsgroepe. Op Dag 0 is die diere deurmekaar in die drukgang gejaag en behandel in ooreenstemming met hul ewekansige toedeling.

Daar was 4 soorte alfametrienbevattende plastiekplaatjies: grys plaatjies met 30% alfametrien, groen plaatjies met 20% alfametrien, en rooi plaatjies en bandjies met 27% alfametrien. Die oorplaatjies was ongeveer 36 cm^2 groot en die bandjies ongeveer 80 cm^2. Die diere is verdeel in die volgende behandelingsgroepe:

a) onbehandelde kontrole,
b) een grys plaatjie in elke oor,
c) een groen plaatjie in elke oor,
d) een rooi plaatjie in die linkeroor plus 'n rooi bandjie om die stertkwas, en
e) een rooi plaatjie in elke oor plus 'n rooi bandjie om die stertkwas.

Bosluistellings

Die bosluise is getel op Dag 1,2,3,4,5,7,14 en 21.

Om die bosluise op elke bees te tel, is die diere in 'n drukgang geplaas en die koppe in 'n nekklem geïmobiliseer. Nadat die bosluise in die ore, onder die stert, op die melkspieël en op die rug en sye getel is, is die dier op 'n kanteltafel neergetrek sodat die bosluise in die oksels, op die pens en in die lieste getel kon word. Alle bosluise is met die blote oog getel op hul voorkeurplekke op die dier se lyf as volg: bontbosluis *(Amblyomma hebraeum)* op die dele bedek deur dun vel, nl. die oksels, pens, lieste, melkspieël en perineum; bruin oorbosluis *(Rhipicephalus appendiculatus)* in die ore en rooipootbosluis *(Rhipicephalus evertsi evertsi)* op die perineum. Bloubosluise *(Boophilus decoloratus)* is oor die dier se hele lyf getel.

Tydens die telproses is die bosluise onderskei op grond van geslag (in die geval van *A. hebraeum)* en graad van versadiging *(A. hebraeum* wyfies en *Rhipicephalus* spp) en geklassifiseer as plat, half- en volgesuig. *Rhipicephalus* spp mannetjies en plat wyfies is saam getel.

Volsuigende *B. decoloratus*-wyfies groter as 4,5 mm is afsonderlik getel. Alhoewel die veronderstelling nog nie vir hierdie spesie bewys is nie, is daar aanvaar dat hierdie bosluise, soos in die geval van *Boophilus microplus*[10], binne die volgende 24 h sou volsuig en afval.

UITSLAG

Die gemiddelde tellings van *B. decoloratus, R. appendiculatus,* en *R. e. evertsi* word weergegee in Fig 1-3. Fig 4-7 beeld die aantal en verspreiding van *A. hebraeum* op die verskillende behandelingsgroepe skematies uit.

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Ontvang: Mei 1988 Aanvaar: November 1988

Fig 1: **Gemiddelde tellings van bloubosluise** *(B. decoloratus)* **op onbehandelde beeste (kontrole) en beeste met oorplaatjies alleen (grys en groen) of oorplaatjies plus stertbande (rooi)**

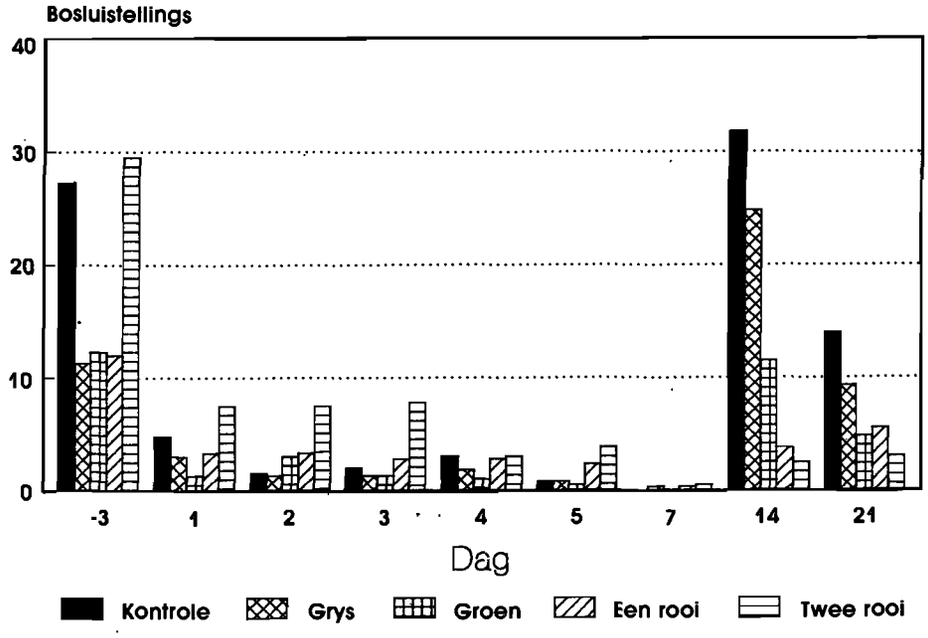
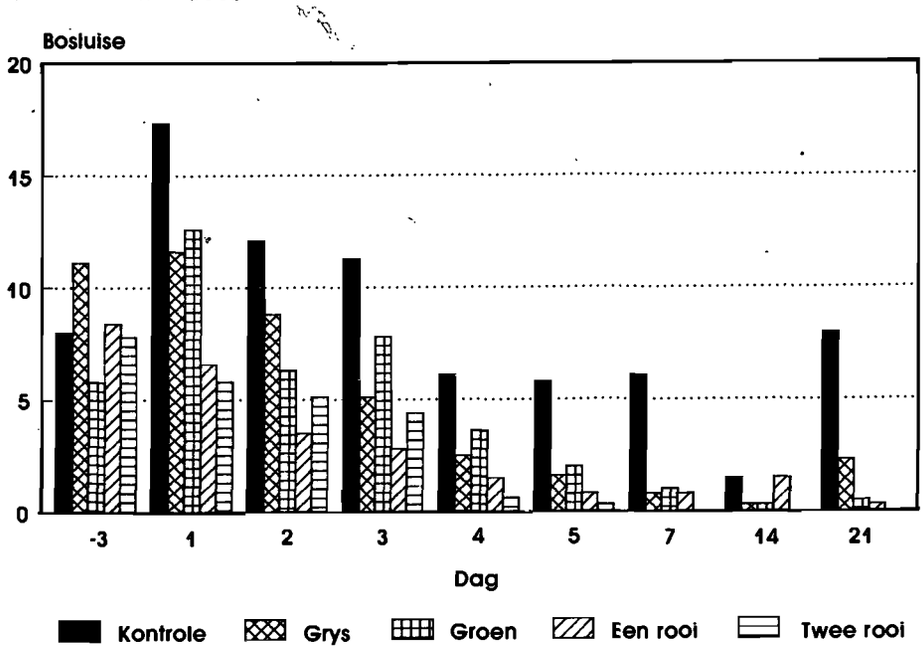


Fig 2: **Gemiddelde tellings van bruin oorbosluise** *(R. appendiculatus)* **op onbehandelde beeste (kontrole) en beeste met oorplaatjies alleen (grys en groen) of oorplaatjies plus stertbande (rooi)**



Gemiddelde tellings van *B. decoloratus, R. appendiculatus,* en *R. e. evertsi* op alle diere het bestendig afgeneem tydens die eerste 7 d na aanwending van die plaatjies. Teen Dag 14 was die bloubosluisgetalle op die onbehandelde beeste weer soos op Dag —3. Bruin oorbosluisgetalle het teen Dag 21 weer begin toeneem. Op die onbehandelde beeste het rooipootbosluise *nie* gedurende die proef na hul getalle van voor behandeling teruggekeer nie, maar het wel daarna teruggekeer in een van die behandelde groepe (grys plaatjies).

Nie een van die behandelings was baie doeltreffend teen bloubosluise nie.

Oorplaatjies alleen was ook nie baie doeltreffend teen die rooipootbosluis nie, maar hul getalle op die perineum is verminder deur die gebruik van 'n stertband.

Die rooi en groen plaatjies was meer doeltreffend teen bruin oorbosluis as grys plaatjies en een rooi plaatjie was oënskynlik netso goed as twee.

Die grys en groen plaatjies het geen uitwerking op bontbosluise gehad nie. 'n Kombinasie van een of twee rooi oorplaatjies plus 'n stertband het egter bontbosluis-getalle doeltreffend verminder.

BESPREKING

Die afname van die gemiddelde tellings van *B. decoloratus, R. appendiculatus,* en *R. e. evertsi* op alle diere was waarskynlik die gevolg van oordraging van alfametrien op die hande van die ondersoekers tydens die bosluistelproses en dui op 'n leemte in die proefuitleg. Tydens die aanwending van die plaatjies op Dag 0 en die bosluistelling of Dag —3 en 1, is die beeste deurmekaar in die drukgang gejaag. Vanaf Dag 2 is sorg gedra om telkens die onbehandelde beeste se bosluise eerste te tel.

Indien daar noemenswaardige afskuur van die aktiewe bestanddeel van behandelde na onbehandelde diere was, soos wel voorkom by proewe met piretroiëde waar verskillende behandelingsgroepe in dieselfde kamp wei, sou hierdie afname meer langdurig gewees het. Die feit dat bosluisgetalle op die onbehandelde beeste na Dag 7 begin herstel het, kan beskou word as 'n aanduiding dat die vermoedelike besoedeling nie die beoordeling van die plaatjies se doeltreffendheid oor die proeftydperk verhinder het nie. Dit is egter raadsaam om by enige proef met 'n piretroïed, kruisbesoedeling tydens hantering van die diere te vermy en die behandelingsgroepe apart aan te hou. Indien dit gedoen word, moet sorg gedra word dat die groepe aan dieselfde bosluisbesmetting blootgestel word.

Die doeltreffendheid van 'n piretroïedeurweekte voorwerp is waarskynlik nie slegs afhanklik van die totale hoeveelheid aktiewe bestanddeel nie, maar ook van die vrystellingstempo wat die konsentrasie in die haarkleed sal bepaal. In ons proef was die rooi materiaal oënskynlik 'n beter medium (moontlik omdat daar meer daarvan was: 1 of 2 oorplaatjies plus 'n stertband). Aan die ander kant was die grys plaatjies ondanks die effens hoër alfametrieninhoud waarskynlik minder doeltreffend as die groen plaatjies vanweë swakker vrystelling.

Deurweekte oorplaatjies word geruime tyd al oorsee gebruik vir die beheer van 'n verskeidenheid ektoparasiete van beeste. Die gewildste aanwending is vir die beheer van gesig- en horingvlieë, maar dit is ook al gebruik teen *Amblyomma maculatum,* wat op groot soogdiere se ore voorkom[1 2 4], en ander bosluise met die oog op bosluisoorgedraagde siektebeheer[9].

In Suid-Afrika is deurweekte oorplaatjies al doeltreffend gevind teen bruin oorbosluise[7]. Die probleem is dat hierdie bosluis selde alleen op beeste/ voorkom en meesal vergesel word van ander bosluise wat wyd oor die bees se lyf versprei is, soos bont- en bloubosluise[6]. Dit bemoeilik beheer deur middel van oorplaatjies alleen[7], soos ook aangetoon in ons proef. Slegs waar 'n deurweekte stertband (saam met oorplaatjies) gebruik is, is daar bevredigende beheer van bontbosluise en rooipootbosluise verkry. Daar was geen verskil tussen die groepe met 1 of 2 rooi plaatjies in die beheer van *A. hebraeum* nie.

Swak beheer van bosluise wat aanheg op ander liggaamsdele as die kop soos *Ixodes ricinus*[8], is oorsee ook al beskryf, en daar is ook gevind dat doeltreffendheid verbeter indien 'n stertband saam met oorplaatjies gebruik word. Dit is onbekend of hierdie verbetering te danke is aan die vermeerdering in die hoeveelheid piretroïed, of die beter verspreiding van die aanwendingspunte.

Fig 3: **Gemiddelde tellings van rooipootbosluise** *(R. e. evertsi)* **op onbehandelde beeste (kontrole) en beeste met oorplaatjies alleen (grys en groen) of oorplaatjies plus stertbande (rooi)**

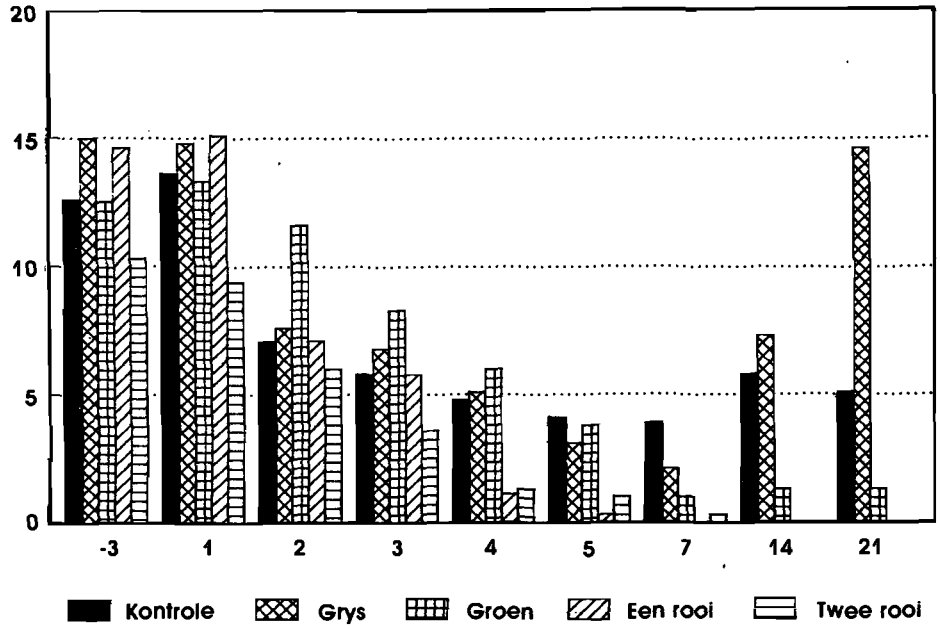


Fig. 4: *Amblyomma hebraeum* **op Dag 21 op onbehandelde beeste. Een kolletjie stel 5 bosluise voor.**

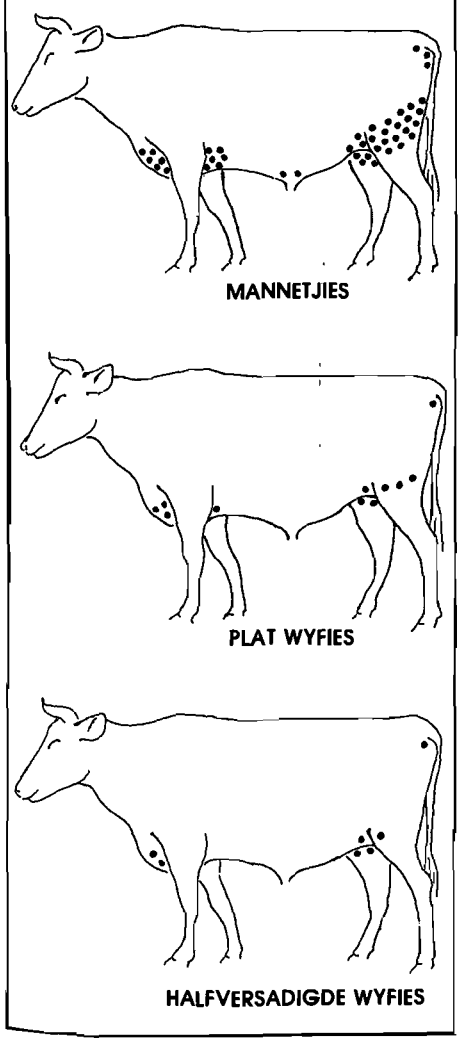


Fig. 5: *Amblyomma hebraeum* **op Dag 21 op beeste met grys oorplaatjies. Een kolletjie stel 5 bosluis voor.**

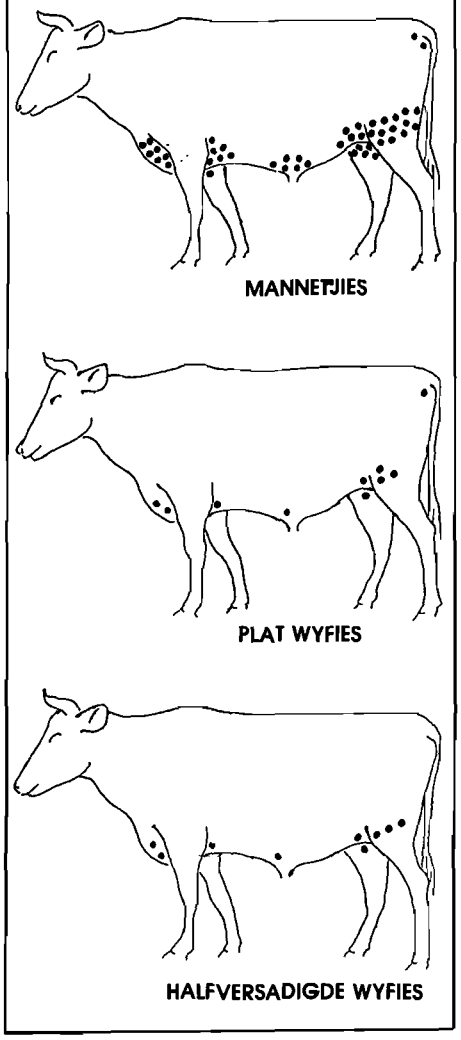


Fig. 6: *Amblyomma hebraeum* **op Dag 21 op beeste met groen oorplaatjies. Een kolletjie stel 5 bosluise voor.**

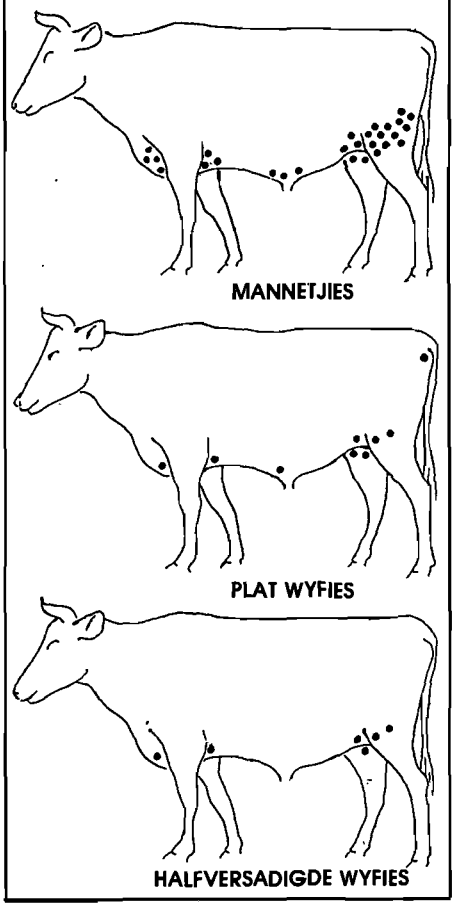
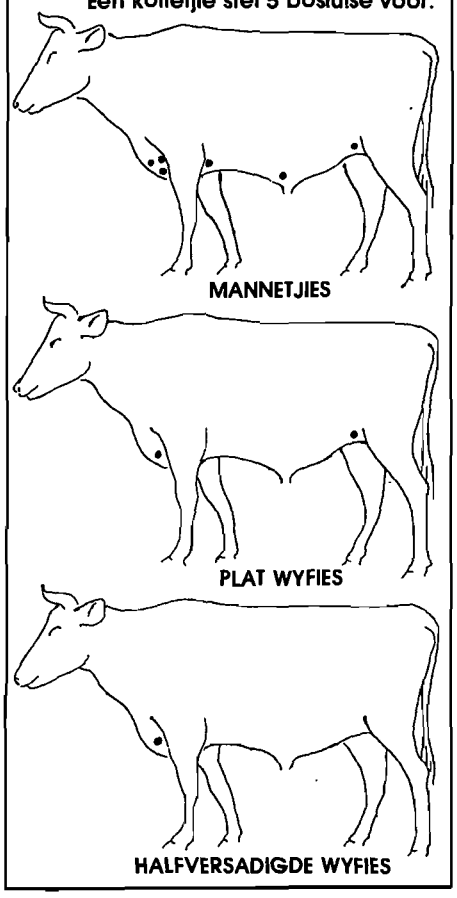


Fig. 7: *Amblyomma hebraeum* **op Dag 21 op beeste met twee rooi oorplaatjies en 'n stertband. Een kolletjie stel 5 bosluise voor.**



of stertbeweging, of 'n kombinasie van hierdie faktore.

Daar is in hierdie proef nie gekyk na die uitwerking van die behandeling op onvolwasse bosluise nie. Dit is onmoontlik om 'n betroubare aanduiding van getalle op lewende diere te kry (ons beskou skattings met 'n subjektiewe waarde wat wissel van "+ tot +++" en wat gegrond is op ondersoek van die pote en ore as nutteloos). In laboratoriumproewe is larwes gewoonlik gevoelig vir laer konsentrasies van dieselfde aktiewe bestanddeel as volwassenes (Schröder J & Ford A A, ongepubliseerde waarnemings). Ons aanvaar dat doeltreffendheid teen volwasse bosluise op beeste, doeltreffendheid teen die onvolwassenes ook impliseer.

Stertbande heg moeiliker vas as oorplaatjies en die grootste probleem wat ondervind is, was om die bandjie op so 'n wyse aan te heg dat dit nie later afval nie. Daarom is van die sterthare om die bandjie gevleg en met kontakkleefstof vasgeplak. Sorg moet egter gedra word om nie die bloedvoorsiening te belemmer nie[8].

'n Kombinasie van 'n stertband en oorplaatjie sou waarskynlik vir die beesboer 'n aanvaarbare alternatiewe beheermetode wees indien dit slegs een maal per seisoen aangewend hoef te word, vanweë die arbeid verbonde aan die aanwending. Dit sou dus 'n nawerking van 4 tot 6 maande (afhangend van die landstreek en bosluisbesmetting) vereis. Die materiaal wat ons getoets het sou nie aan hierdie vereiste voldoen nie.

DANKBETUIGING

Die tegniese hulp van Mnre D N Odendaal en D H Swift, Mej E F Hardman en Mev A A Ford, word met dank erken.

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Book review/Boekresensie

MILLER'S GUIDE TO THE DISSECTION OF THE DOG

HOWARD E EVANS and ALEXANDER DE LAHUNTA

3rd Edn. W B Saunders Company, Philadelphia, PA 19105. 1988 pp SVII and 361, illustrations 233 and 5 tables. Price £14-50 (ISBN 0-7216-2323-9)

This guide facilitates a thorough dissection of the dog and is intended for veterinary students and small-animal practitioners. The third edition is more comprehensive than the second edition and has more illustrations. The nomenclature is based on the Nomina Anatomica Veterinaria (3rd edn. 1983) and anglicised terms are used.

In the introduction the authors discuss some anatomical terminology and directional terms. The following section deals with the skeletal and muscular systems. The bones, muscles and joints of the thoracic and pelvic limbs, the bones and joints of the axial skeleton and the muscles of the trunk are discussed.

In the next three sections the blood vessels, nerves and splanchnology of the different body regions and organ systems are dealt with. In the last section a detailed description of the nervous system with its blood supply is given.

A logical sequence is followed, the text being accompanied by excellent illustrations. This book will be of great value to pre-and post-graduate veterinary students and although primarily a dissection guide, the illustrations and theoretical content are such that the book will prove an asset in any small-animal practice.

H B GROENEWALD

THE MALE REPRODUCTIVE PATTERN AND HISTOLOGY OF THE TESTES OF THE LESSER YELLOW HOUSE BAT, *SCOTOPHILUS BORBONICUS* (E GEOFFROY, 1803) (CHIROPTERA: VESPERTILIONIDAE)

N J VAN DER MERWE* and I L RAUTENBACH+

ABSTRACT:
Monthly samples of the testes of the vespertilionid bat, *Scotophilus borbonicus* were collected, sectioned and examined by light microscopy. Spermatogenesis was prolonged and extended over 8 months, coinciding with spring, summer and autumn. During any particular month only certain spermatogenic cells are present in the seminiferous tubules. The mediastinum is, unlike that of most other mammals, situated towards the caudal border of the testis. This species is eminently suited for the study of the process of spermatocytogenesis.

Key words: Reproduction pattern, histology, testes, lesser yellow house bat, *Scotophilus borbonicus*

Van der Merwe N.J.; Rautenbach I.L. The male reproductive pattern and histology of the testes of the lesser yellow house bat, *Scotophilus borbonicus* (E. Geoffroy, 1803) (Chiroptera: Vespertilionidae). *Journal of the South African Veterinary Association* (1989) 60 No. 2, 83-86 (En) Department of Anatomy, Faculty of Veterinary Science, Private Bag X04, 0110

INTRODUCTION

In non-hibernating bat species, the male and female reproductive processes are more or less synchronised so that initially spermatogenesis and follicular development, and subsequently ovulation and fertilisation, coincide.

In the aseasonally polyestrous species, spermatogenesis and accessory genital gland activity continue throughout the year[5] e.g. *Rousettus aegyptiacus*[6]. In the bimodally polyestrous species it would appear that the males are capable of inseminating the females at any time of year, but females are only able to support two pregnancies and lactations per year[2].

The different non-hibernating members of the Vespertilionidae, display all the abovementioned patterns of reproduction, and females may even store sperm for prolonged periods in their reproductive tracts e.g. *Scotophilus wroughtoni*[4] and *Nycticeius schlieffenii*[10].

Scotophilus borbonicus is a non-hibernating vespertilionid bat with an easterly distribution in the subtropical regions of subsaharan Africa. Ovulation, copulation and fertilisation occur in autumn (end of March - April) and the gestation period is 8 months. Twins are the rule. This paper describes the male reproductive pattern as well as the histology of the testis.

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Received July 1988 Accepted: November 1988

MATERIALS AND METHODS

Specimens of *S. borbonicus* were collected monthly (September 1984 — August 1985) with macro-mistnets[7] at several localities at Pafuri (22° 25'S;31° 12'E) in the northern region of the Kruger National Park in the eastern Transvaal Lowveld. Since conservation rules prohibit too many samples being collected, the objective was to acquire only 5 male specimens during each calender month.

At the field laboratory the animals were killed with ether, and the reproductive organs were removed. The testes were preserved in 10% phosphate buffered formalin after the tunica albuginea had been slit. After a minimum period of 4 d of fixation, the testes were dehydrated by means of a graded sequence of alcohols, cleared in xylol and embedded in paraffin wax (Histosec, Merck). The embedded testes were sectioned at 5 μm using a Reichert sliding microtome. The sections were stained, using the standard Mayer's haematoxylin and eosin technique. Seminiferous tubule diameters were quantified by measuring 2 diameters at right angles in cross sections of 10 seminiferous tubules per testis. The measurements were made with an ocular micrometer.

All specimens studied were deposited as voucher specimens in the National Research Collections of the Transvaal Museum. Individual specimens were subsequently checked on skull features for correct identifications, and for relative age. Although the chronological age at which reproduction is commenced has not been determined, it was found that all young adults had been reproductively active[11]. Hence care was taken in the field to select only adult specimens, and tooth eruption and wear were studied in the laboratory to ensure that all the samples collected were fully matured individuals.

RESULTS

The parenchyma of the testis of the lesser yellow house bat is enclosed in a fibrous capsule, the tunica albuginea. Towards the caudal aspect, a thickening of the tunica albuginea, extending about halfway into the gland, forms the mediastinum (Fig. 1). Thin collagenous septa, the septulae testis, radiate from the mediastinum and join the tunica albuginea dividing the testis into lobules (Fig. 1). Within these lobules the convoluted seminiferous tubules are situated, the latter lined with a stratified germinal epithelium. The germinal epithelium is supported by Sertoli cells. The latter cells rest on the basement membrane of the seminiferous tubule. Within the mediastinum a labyrinthine plexus of epithelial lined spaces, the rete testis, is situated. The seminiferous tubules are joined to the rete testis by straight tubules, the tubuli recti. The latter as well as the rete testis are both lined with cuboidal to columnar epithelial cells.

The angular spaces between the seminiferous tubules contain clumps of polygonal cells, the interstitial cells of Leydig (Fig. 2, 3 & 4). As a result of changes in the activity of these cells, their appearance varies at different times during the year (see below).

The blood vessels supplying the parenchyma of the testes are situated on the inner aspect of the tunica albuginea, forming the tunica vasculosa from which numerous smaller vessels penetrate to supply the testis.

Spermatogenesis follows a cyclic pattern as can be seen from the changes in mean monthly diameter of the seminiferous tubules (Fig. 9). From July to December the mean diameter gradually increases. This is followed by a sharp increase up to April, when copulation occurs. Thereafter a sharp drop occurs in May, followed by a more gradual decrease to the low values of July.

The cyclic changes in the diameter of the tubule coincide with the onset and cessation of spermatogenesis. During winter (May — August) the germinal epithelium consists mainly of Sertoli cells and a few scattered spermatogonia (type A) (Fig. 2) and has the appearance of a pre-pubertal testis. In September (spring) mitotic activity in the spermatogonia commences and the testes enlarge concurrently. The spermatogonia, which are situated alongside the basal laminae of the seminiferous tubules, undergo division, resulting in type A and B spermatogonia (Fig. 3).

Specimens collected at the end of October exhibited a marked increase in the thickness of the germinal epithelium so that the volume of the seminiferous tubule lumina was dramatically reduced

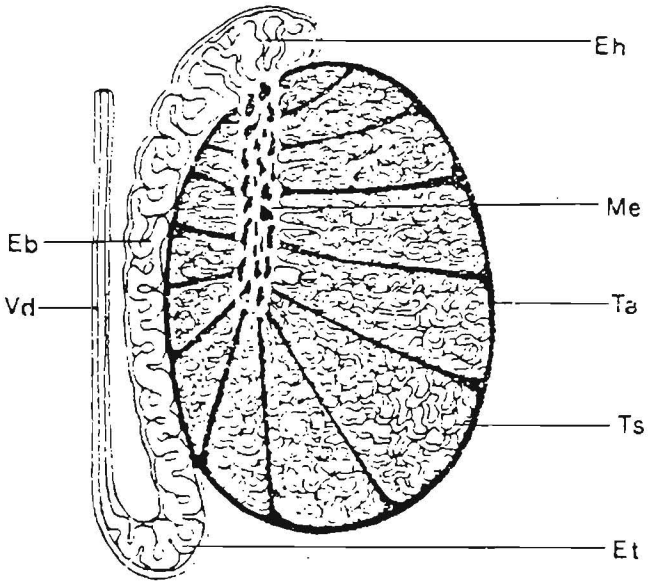

Fig. 1: **A diagrammatic section through a testis to illustrate the internal anatomy. Eb = body of epididymis; Eh = head of epididymis; Et = tail of epididymis; Me = mediastinum testis; Ta = tunica albuginea; Ts = tubuli seminiferi contorti with the tubuli seminiferi recti as the connecting pieces between the former and the mediastinum testis; Vd = ductus deferens. Note that the mediastinum testis is situated eccentrically**

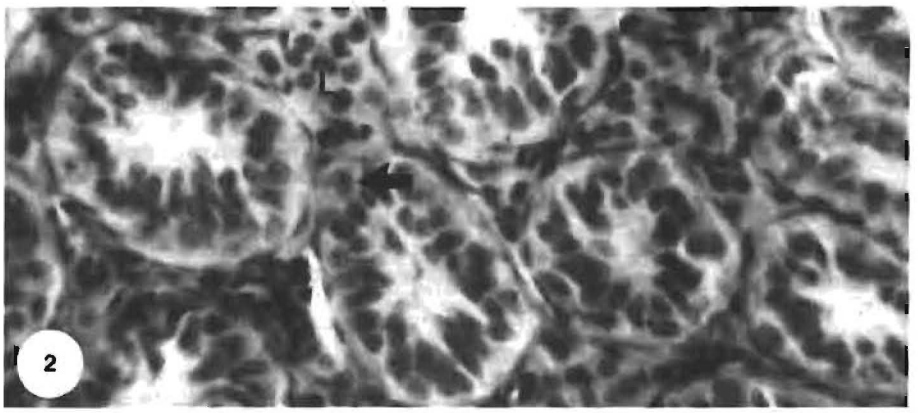

Fig. 2: **Typical appearance of the testis in the inactive condition (May to August). The seminiferous tubules consist of Sertoli cells and a few spermatogonia (type A) (arrow). Leydig cells - L. X 400**

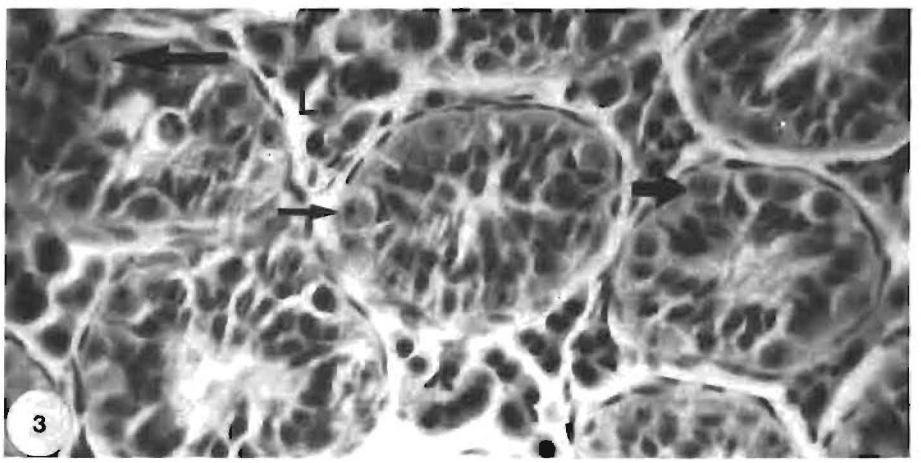

Fig. 3: **A photomicrograph of the seminiferous tubules of a testis collected towards the end of September showing more spermatogonia of Type A (small arrow). A few of type B (medium arrow) and a mitotic figure (large arrow), indicating commencement of proliferation. Leydig cells - L. X 400**

(Fig 4). This is due to a marked increase in mitotic activity. At the end of November some fluid is secreted into the seminiferous tubule lumina and at the end of December the first primary spermatocytes appear (Fig 5).

Seminiferous tubules of specimens collected at the end of January and February exhibited primary and secondary spermatocytes. The primary spermatocytes exhibited the largest nuclei of the spermatogenic cells (Fig. 6) At this stage, spermatogonial mitotic activity is at a very low level.

Specimens collected at the end of March showed numerous developing spermatids with an occasional spermatogonium present (Fig. 7). At the end of April the majority of seminiferous tubules showed spermatozoa in the lumina (Fig. 8).

The Leydig cells also exhibit an annual cyclic pattern. During winter the nucleus is small, condensed and stains basophilically. The cytoplasm is more eosinophilic and less vacuolated when seen in paraffin sections during winter (Fig. 2) than in spring and summer. During spring and summer the nucleus is enlarged, with a prominent nucleolus. At this time, the cytoplasm appears finely vacuolated in sections, due to increased steroid secretion. (Fig. 4).

DISCUSSION

The macro- and microstructures of the testes of *S. borbonicus* conform closely to the general mammalian pattern, although the position of the mediastinum corresponds with that of *Homo sapiens*[1] and the domestic horse[3].

In non-hibernating bats ovulation, spermatogenesis, copulation and fertilisation usually take place concurrently[5]. There are however exceptions to this trend, such as in *Miniopterus australis* where spermatozoa are stored in the epididymis for several weeks after involution of the testes[8] and fertilisation only occurs several weeks after the latter event.

The male reproductive pattern in the lesser yellow house bat is closely synchronised with that of the female and spermatogenesis reaches its peak when the females ovulate during April. During winter, the testes are involuted. With increasing day length, gonadotropin production is apparently increased with a resultant increase in Leydig cell activity[9]. The increase in androgen production, in conjunction with follicle stimulating hormone, initiates spermatogenesis.

Another contributing factor to this reproductive pattern appears to be the availability of insects. During winter, insect numbers are low and the accumulated fat reserves of the males become progressively depleted (unpublished data). In spring and early summer, higher energy intake is presumably initially utilised for the restoration and maintenance of body functions, resulting in the extension of spermatocytogenesis. The process of spermiogenesis, however, does not appear to be delayed

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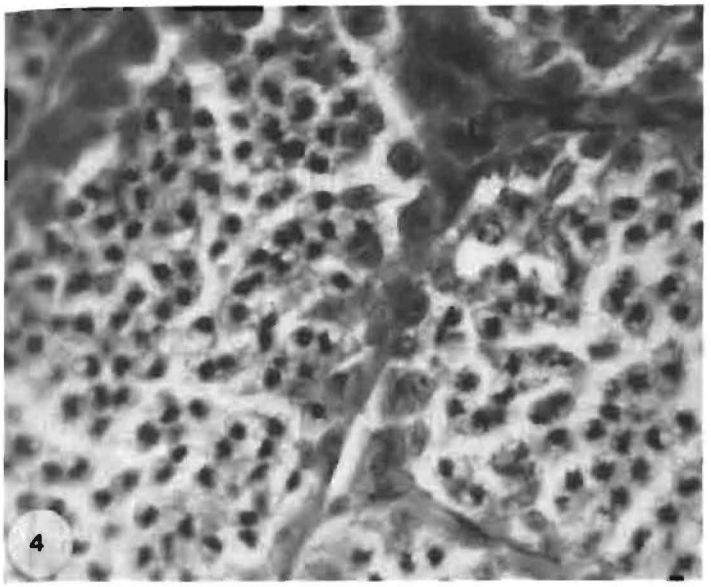


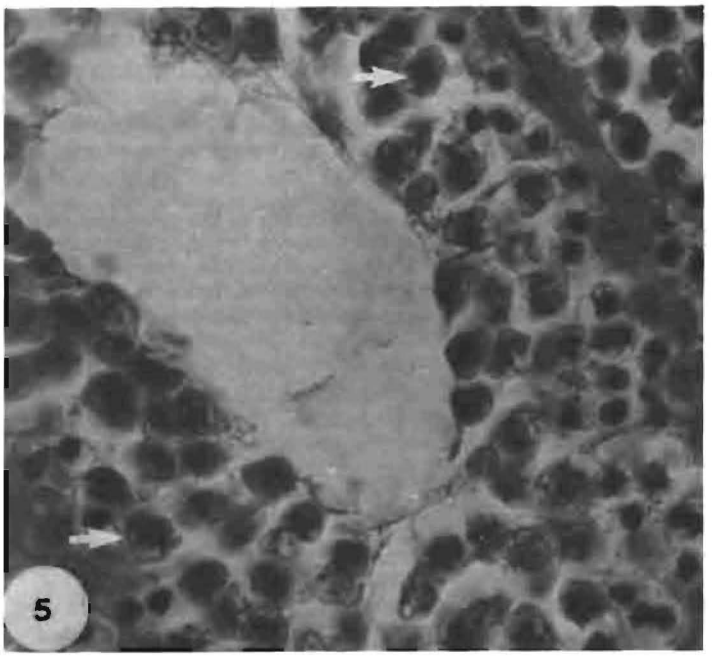
Fig. 4: A photomicrograph of a section of a testis collected in October. Note the marked proliferation of spermatogonia with the subsequent filling of the lumen. Spermatogonia of type B are mostly present. Accumulation of steroids in Leidig cells (L) (seen as vacuoles) is more prominent. The large nuclei at the peripheri of the seminiferous tubules are mostly Sertoli cell nuclei. X 400

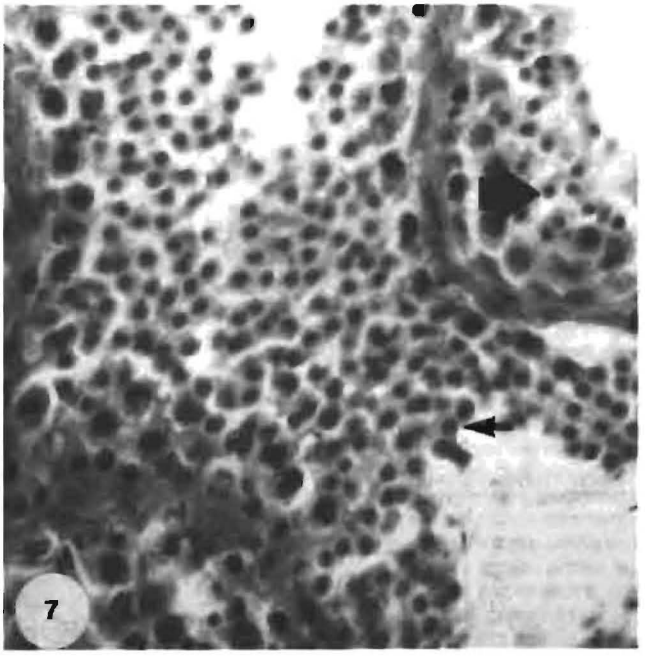
Fig. 5: This photomicrograph illustrates the appearance of primary spermatocytes (arrow) in the seminiferous tubules of a specimen collected towards the end of December. X 400

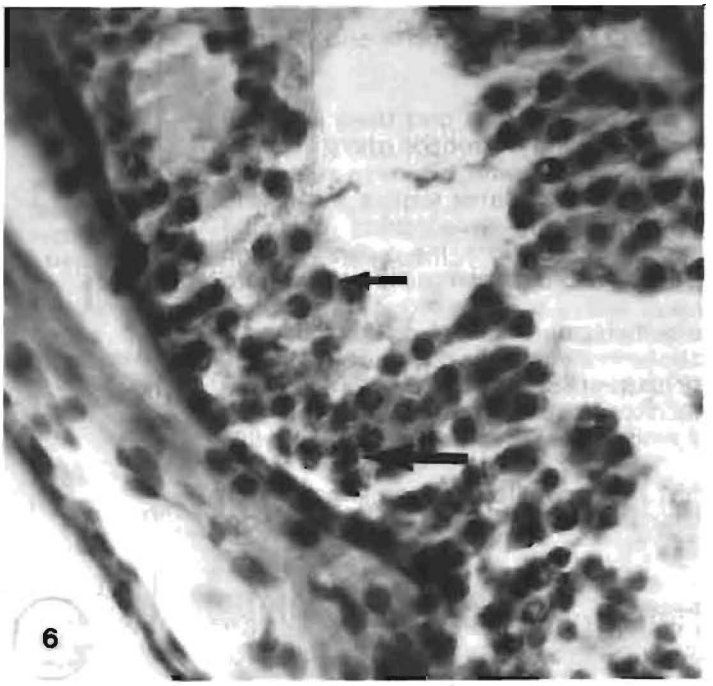
Fig. 6: Photomicrographs illustrating the appearance of secondary spermatocytes, (small arrow) and a few primary spermatocytes (large arrow). X 400

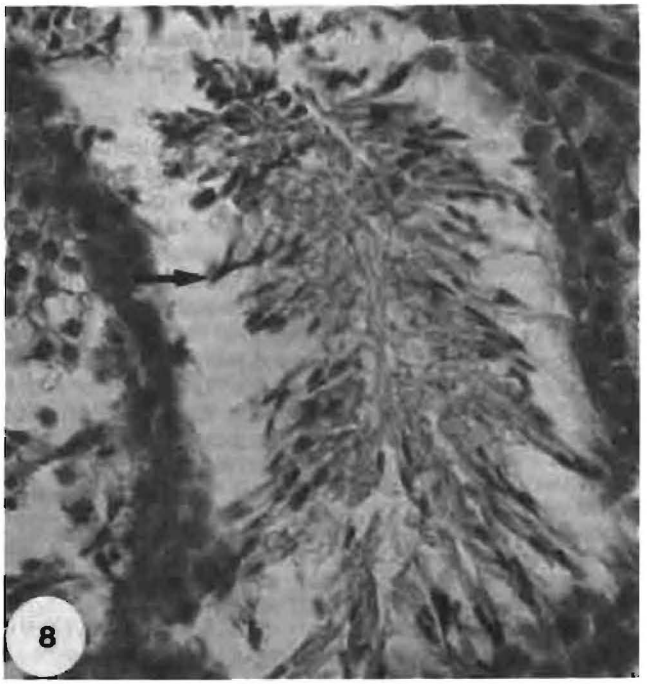
Fig. 7 A photomicrograph of a section showing spermatids (small arrow) and developing spermatids (large arrow) end of March). X 100

Fig. 8: A photomicrograph of a section of a testis collected at the end of April showing spermatozoa (arrow) in the seminiferous tubules. X 200









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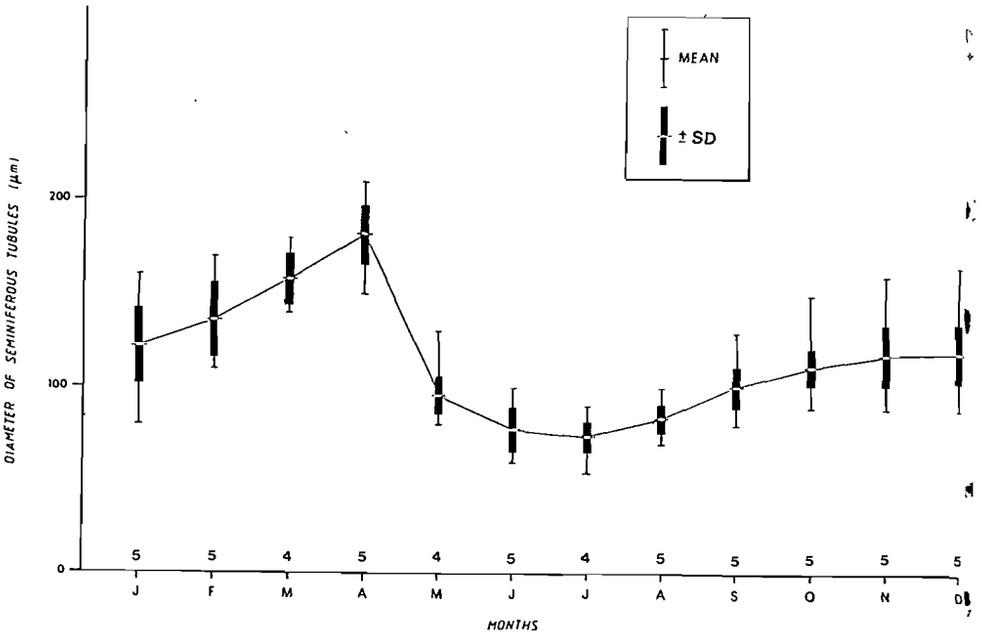

Fig. 9: **Graph illustrating the monthly and annual variations in the diameter of the seminiferous tubules. For each monthly sample, the observed ranges are indicated by a vertical line, the mean is indicated by a horizontal line bisecting the vertical line, and plus or minus one standard deviation of the mean is indicated by a solid rectangle. The numbers indicate the total number of specimens examined each month**

Book review/Boekresensie

THE METABOLIC PROFILE TEST

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Oxford University Press, Walton Street, Oxford OX2 6DP, UK. 1987 pp IX and 179, one table, Price RSA R129-10 (ISBN 0-19-854544-4)

This book, set out in 3 sections (Background, Components and Uses) is objective, informative and up-to-date. Each chapter (1 for the first section and 9 each for the last 2 sections) is logically subdivided, provided with extensive reference lists and ends with a sub-heading "Conclusions". This latter feature makes the book an ideal reference book for the practising veterinarian and the veterinary/agricultural research scientist. In the conclusions, the authors pull no punches and clearly indicate the proven applications as well as the areas where more work is required.

It is a pity that the title suggests that the authors only intend to address the well-known issues surrounding the British Metabolic Profile Test for dairy cattle, designed by them in 1971. The fact that there are chapters on exercise monitoring in the horse and dog, the detection of congenital disorders and applications in fish, wild animals and poultry, make this a work of much broader relevance than the title gives it credit for.

The logical target audience of this book would include herd-health veterinarians, large animal practitioners, nutritionists, veterinary laboratory diagnosticians and researchers in allied fields.

F REYERS

PHYSIOLOGICAL AND BLOOD BIOCHEMICAL RESPONSES TO SUBMAXIMAL TREADMILL EXERCISE IN CANAAN DOGS BEFORE, DURING AND AFTER TRAINING

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ABSTRACT:
Physiological haematological and blood biochemical parameters were studied in Canaan dogs (n = 8) as possible indices of fitness. These parameters were then used to distinguish between fit and unfit dogs and to monitor the changes in these parameters during training and detraining periods. Fitness was defined as the ability to run for one hour on a motorised treadmill (speed 8,65 km h^{-1}, inclination 10°) while maintaining rectal temperature and heart rate below 41°C and 250 beats min^{-1} respectively. Fit dogs showed consistently lower values for heart rate and rectal temperature during exercise and recovery, and significantly lower increases for post exercise plasma creatine kinase activity and plasma lactate concentration. Significantly higher values for haemoglobin concentration were found immediately post exercise. There were no significant differences between fit and unfit dogs for post exercise plasma concentrations of aspartate aminotransferase, white blood cell count or total protein, although the unfit dogs showed a tendency towards higher values. Similarly, unfit dogs tended to have lower values for plasma glucose concentration, haematocrit and red cell count. Values for heart rate and rectal temperature during exercise differed significantly between various dogs until numbers fell, due to the inability of certain dogs to complete the exercise test. Dogs were consistent according to whether their values lay above or below the mean value for all dogs. These differences disappeared after 8 weeks of training. Fitness deteriorated after 3 to 5 weeks of detraining. According to these results, heart rate and rectal temperature appear to be the most suitable and sensitive indicators of fitness in tracking dogs. The haematological and blood biochemical parameters tested were found to be of limited use. It is suggested that apparent inherent superiority of dogs should be accounted for in prospective breeding programmes.

Key words: Dogs, treadmill, exercise, parameters of fitness

Sneddon J.C.; Minnaar P.P.; Grosskopf J.F.W.; Groeneveld H.T. Physiological and blood biochemical responses to submaximal treadmill exercise in Canaan dogs before, during and after training. *Journal of the South African Veterinary Association* (1989) 60 No. 2, 87-91 (En). Department of Physiology, Faculty of Veterinary Science, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

Since working dogs usually have to be kept in kennels, their lack of fitness has been found to be an important limiting factor in their performance. This study was therefore designed to establish parameters of fitness and to apply the parameters to dogs at work during unfit, in training, fit and detraining periods.

Canaan dogs have been described as intelligent and hardy animals when used for tracking in arid areas[10 14], but no experimental documentation of their performance in this regard exists.

Astrand[3] defined physical fitness as "the ability of the organism to maintain various internal equilibria as closely as possible to the resting state during strenuous exertion, and to restore promptly after exercise any equilibriums that have been disturbed". Parameters that could serve as indices of fitness were chosen in the light of this definition.

Submaximal values in working heart rate have been successfully used to discriminate between fit and unfit dogs[13 15 16]. The same holds true for rectal temperature[4 13 15]. Dogs exhibit splenic contraction during exercise[9] with resultant increases in circulating erythrocytes and white cell count. Increasing fitness has also been associated with an increase in blood volume[8] and an increased oxygen carrying capacity of blood[2 16].

Quantitative plasma measurements of enzyme release from hypoxic skeletal muscle in healthy dogs have been used to estimate the degree of local hypoxia caused by muscle exertion[5 6 7 9]. The enzymes commonly used are creatine kinase (CK) and aspartate aminotransferase (AST). Plasma lactate concentration has also been used for this purpose[13 15 16]. Total plasma protein concentration (TP) has been used as an index of dehydration in exercising dogs[9]. Plasma glucose concentration may be used as an index of cortisol metabolism and glycogen storage capacity in muscle[12].

MATERIALS AND METHODS

Experimental animals and design

Canaan dogs (n=8, 2 males and 6 females), a 2-year-old and the remainder aged 5-7 years, mass 15-20 kg (one 25 kg) were individually housed in cages (0,85 x 0,85 x 1,38m^3) in a ventilated room with an ambient temperature of 15 ± 5°C. These dogs were offspring of a pair of Canaan dogs from Israel and were therefore closely related. As these were the only Canaan dogs available, a more uniform group in respect of age, sex and weight could not be selected. Dry commercial dog food was provided in a quantity sufficient to maintain body mass. Water was provided ad lib. The experiment consisted of 5 phases, and lasted from May to September 1986 during the Transvaal winter when temperature and relative humidity remain fairly constant (15 ± 3°C, 45 ± 9%, The Weather Bureau, PB X447, Pretorius St, Pretoria).

During the first (cage-confined) phase, the dogs (A - H) were confined to cages for one month prior to a subsequent experimental period, in an attempt to familiarise them with their experimental environment, and to achieve a state of unfitness[12]. For the duration of the experiment, blood samples were drawn from a jugular vein at 08h00 and 15h00 daily (Monday to Friday), to provide baseline information on the parameters under investigation.

During the second (unfit) phase, each dog completed 4 treadmill runs spread over a 2-week period, at a steady jog (speed 8,65 km h^{-1}, inclination 10°). Exercise continued until severe fatigue was evident (rectal temperature > or = 41°C and/or heart rate > 250 beats min^{-1} and/or the dog dropped to the back of the treadmill)[15]. This was judged equivalent to the work load experienced by tracking dogs in the field (S. Elliott 1986, SADF, Dog Centre, Bourke's Luck 1272 R.S.A.). Blood samples were drawn from a jugular vein before exercise and during

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Received: April 1987 Accepted: January 1989

recovery, and were analysed for selected blood biochemical and haematological parameters (see below).

During the third (in training) phase, the dogs were subjected to 8 weeks of treadmill exercise (speed 8,65 km h^{-1} inclination 10°), until severe fatigue was evident (rectal temperature > or = 41°C and/or heart rate > or = 250 beats min^{-1} and/or the dog dropped to the back of the treadmill)[15]. After this period, all the dogs were judged to be fit (capable of running for one hour while remaining within the previously-mentioned limits of fatigue). Blood samples were drawn from a jugular vein before and immediately after exercise, and were analysed as indicated above.

During Phase 4, the fit dogs were subjected to the exercise described for Phase 2.

During Phase 5 (detraining), the dogs were split into random pairs using random number tables, and were cage-confined as described previously. The first pair was run after 3 weeks of cage confinement, and the remaining pairs after 5,7 and 8 weeks of cage confinement[12]. The exercise test employed during the second phase was repeated in order to assess whether fitness had deteriorated significantly during 8 weeks of cage confinement. Blood and physiological parameters were monitored as described for Phase 3.

Analytical techniques

Heart rate was monitored at rest, at each 5 min interval during exercise and at 5 min post exercise. A telemetric device was used (Mennen Greatbatch Electronics Inc, PO Box 874 Wendywood 2144 R.S.A.), in conjunction with skin-adhesive electrodes (Macmed, PO Box 43333 Industria 2024, R.S.A.) and a stockinette (Smith & Nephew Ltd, Box 15768 Doornfontein, R.S.A.) to immobilise the electrode leads so that a trace, free of interference could be obtained. Lead 1 of Einthoven's triangle was used. The telemetric device was also earthed to the treadmill to cut down on electrical noise from the treadmill motor. Rectal temperature was monitored at rest and at 1 min intervals during exercise, using a thermistor probe placed in the rectum to a depth of 10 cm (Kane-May Measuring Instruments, Burrowfield, Welwyn Garden City, Herts AL7 4TU, England).

Haematocrit (Ht), haemoglobin (Hb), red cell count (RCC) and white cell count (WCC) were measured using standard methods (Coulter Electronics Ltd, Harpenden, Herts, England). Total plasma protein (TP) was measured by the refractometer method[16]. Plasma concentrates of glucose, lactate, creatine kinase (CK) and aspartate aminotransferase (AST) were measured with an automated analyser (Ames Pacer, Ames Division, Miles Laboratories Inc, PO Box Elkhart Indiana 515, U.S.A.), in conjunction with commercial reagent kits (Boehringer Mannheim SA Pty Ltd, PO Box 51927 Randburg 2125 R.S.A. (GOD-PAP 16639, Lactate TC 256773, GOT opt Autopack 258784) and from Worthington Coopers Biomedical Diagnostic Division, Freehold, N J 07728 U.S.A. (CK-NAC 16 25954). All parameters mentioned above were sampled at rest Oh, 20 min, 3h and 24h after exercise, except plasma activities of AST which were measured at 1h and 24h after exercise. The exzymes were measured at a temperature setting of 37°C. All blood samples were drawn from a jugular vein (alternating both sides to minimise damage) using 5 ml evacuated tubes (Venoject Terumo Corporation, PO Box 9171 Johannesburg 2000, R.S.A.) These tubes contained heparin, but not so those used to determine lactate concentration, where plain tubes containing 25 ul Anderson's anticoagulant solution[2] were used.

Statistical methods

Results were analysed using Statistical Analysis Systems computer packages (SAS Institute, Box 8000 Cary, North Carolina 27511-8000, U.S.A.)

Differences between means were accepted as statistically significant at probability levels of $p < 0,05$. Normal statistical tests were applied to the data where corresponding sampling times at different phases were compared, as sufficient data was collected to assume a normal distribution of data within a sampling interval (central limits theorem)[11].

Data from the first phase was subjected to an analysis of variance test to distinguish between variance due to dog and effect of varying time of sampling. A Least Significant Difference test (Tukey's Studentised Range) provided information on differences between dogs. In the absence of significant between-dog differences, parameter values were quoted as ranges.

The second (unfit) and fourth (fit) phases were compared at corresponding sampling times for absolute values for all parameters. A paired 't' test and Least Significant Difference test were also used to quantitify differences in mean values between dogs.

Table 1: **Baseline ranges for haematological and blood biochemical parameters obtained from dogs confined to cages (Phase 1)**

Parameter	Observations	Range	
		Observed	Expected[a]
Ht	261	0,32-0, 60	0,37-0,55
Hb (g ℓ^{-1})	239	119-202	120-180
WCC (x10^9 ℓ^{-1})	240	7-29	6-15
RCC (x10^{12} ℓ^{-1})	281	3,8-8,4	5,5-8,5
TP (g ℓ^{-1})	244	50-89	53-75
AST (U ℓ^{-1})	253	5-96	<40
CK (U ℓ^{-1})	251	15-117	<70
Lactate (μmol ℓ^{-1})	128	60-879	<500
Glucose (mmol ℓ^{-1})	255	1,89-6,55	3,3-5,5

[a]Reyers, Section Clinical Pathology, Faculty of Veterinary Science, 0110 Onderstepoort. Unpublished data

Table 2: **Difference between fit (Phase 4) and unfit (Phase 2) dogs as indicated by difference between mean ± 2SD values for blood biochemical parameters and Hb**

Parameter	Sampling time (h post exercise)	Mean value for unfit dogs	Mean value for fit dogs	Significance
CK (U ℓ^{-1})	0	100 ± 70	25 ± 20	••
	0,2	150 ± 70	25 ± 20	••
	1	210 ± 80	30 ± 20	••
	3	390 ± 200	40 ± 30	•••
	24	150 ± 70	20 ± 5	•••
Lactate (μmol ℓ^{-1})	0	240 ± 75	150 ± 50	•
Hb (g ℓ^{-1})	0	175 ± 10	190 ± 10	•

For the third (in training) phase, parameter change from rest for each week of measurement was analysed using a non-parametric "Univariate" computer package in conjunction with the Wilcoxon Rank Mean Score test for analysis of between dog differences. The time variable used required that a non-parametric test be used for analysis of the data. The following symbols were used for significance levels in the results: $^{*}p \leq 0,05$ $^{**}p < 0,01$ $^{***}p < 0,001$.

RESULTS

Phase 1 (baseline values)

Observed baseline ranges of all haematology and blood biochemical parameters were within or near established clinically normal ranges for dogs with the exception of CK and AST which showed slightly higher ranges than expected[6] (Table 1). No significant differences were observed between dogs or between different days of sampling.

Phases 2 and 4 (unfit and fit dogs)

Of the blood biochemical and haematology parameters monitored, only CK, lactate and Hb showed significant differences between dogs in the fit and unfit states. Post-exercise values were higher in unfit dogs for CK and lactate. Hb concentration was lower in unfit dogs (Table 2).

Comparison of values for unfit and fit dogs revealed that the physiologic parameters (heart rate and rectal temperature) showed more significant changes between dogs and sampling times than the blood biochemical or haematology parameters (Tables 3,4,5 and 6). In the second (unfit) phase, dogs A,C and H had superior stamina and/or lower values for heart rate and rectal temperature than the remaining dogs. These differences disappeared when the dogs were fit, when all dogs had lower values for heart rate than when they were unfit, as well as showing superior stamina (Tables 3,4,5 and 6). Rectal temperature did not show the same drop that heart rate did between the unfit and fit states.

Phase 3 (in-training)

During Phase 3, significant decreases in parameter change from rest were only seen in the parameters illustrated in Table 7. Non-significant trends were seen for rectal temperature (downward trend) and Hb (upward trend).

Phase 5 (detraining)

As the weeks of Phase 5 (detraining) progressed, time of running before fatigue set in decreased. The most dramatic drop occurred between 3 and 5 weeks of cage confinement, when running time decreased from 60 to 30 min. This was accompanied by corresponding increases in heart rate and rectal temperature (Table 8).

All resting parameter values obtained in Phases 2 to 5 lay within the baseline ranges established in Phase 1.

DISCUSSION

In the light of Astrand's definition of fitness[3], the results from these experiments have identified some useful parameters for assessing fitness of tracking dogs, namely, CK, lactate, Hb, and the physiologic parameters, heart rate and rectal temperature. Physiological parameters appear to be more sensitive for this function than blood biochemical parameters. This is in agreement with work done on horses[1]. Heart rate recovery from exercise should be investigated in more detail[8 15 16]. The lower values obtained for heart rate in fit dogs confirms findings of other authors[15 17]. The results for rectal temperature confirm similar trends observed by other authors[4 13]. Rectal temperature appeared less sensitive than heart rate, although it was useful in helping to distinguish dogs which appeared inherently fitter in Phase 2. The lower values for heart rate and rectal temperature in dogs with apparent inherent fitness, point to a superior cardiovascular system, which could be selected for in breeding programmes.

Increase in fitness appeared to be aided by decreased muscle hypoxia as shown by lower concentrations of CK[5] and lactate. This is supported by former work[13 15 16]. Improved oxygen-carrying capacity of fit dogs was shown by higher concentrations for immediately post-exercise haemoglobin concentration. This supports previous work on greyhounds[9]. Similar, although non-significant trends were seen for RCC and Ht. Deterioration of fitness occurred within 3 to 5 weeks of detraining as reported by Tipton[15].

From the heart rate and rectal temperature results it can be seen that certain dogs may be inherently superior to others, and could thus be selected for in-breeding programmes for tracking dogs.

Fitness (the ability to run for at least 1 hour on a treadmill at 8,65 km h^{-1}, inclination 10°, while maintaining heart rate and rectal temperature below 250 beats min^{-1} and 41°C respectively) was attained by 8 weeks of training. Rectal temperature did not show the same drop that heart rate did between the unfit and fit states of the dogs. This could have been due to longer periods of exercise by the fit dogs under slightly higher environmental temperatures (ambient temperature = 25 ± 5°C).

Fitness diminished over a period of 3 to 5 weeks of cage confinement, in terms of decreased stamina (time of running), and

Table 3: **Heart rate values for unfit (Phase 2) dogs during exercise. Values are expressed as mean ± sem of 4 treadmill runs per dog**

Running time (min)	Mean ± sem all dogs	Dogs above mean value	Dogs below mean value	Significance of differences between unfit dogs
Rest	108 ± 3,2	ABEFGH	CD	ns
10	214 ± 2,6	AEFH	BCDG	**
20	216 ± 2,8	ADFH	BCEG	**
30	222 ± 4,1	EH	ABCG	***
40	213 ± 4,1	AEFGH	BC	ns
50	223 ± 4,6	BFHI	ACG	ns
60	230 ± 5,2	AH		ns
5 min post exercise	138 ± 3,6	AEFG	BCDH	**

Table 4: **Heart rate values for fit (Phase 4) and unfit (Phase 2) dogs during exercise. Values are expressed as the mean ± sem of 4 treadmill runs per dog**

Running time (min)	Unfit	Fit	Significance of difference fit : unfit
Rest	109 ± 3,09	95 ± 3,17	**
10	214 ± 4,70	178 ± 4,61	***
20	217 ± 4,48	178 ± 4,56	***
30	223 ± 5,87	181 ± 5,14	***
40	222 ± 6,71	193 ± 5,66	***
*50	228 ± 11,28	198 ± 6,53	*
*60	138 ± 2,80	126 ± 2,97	**
5 min post exercise			

* Dogs A and H only completed over 50 min of running when unfit

Table 5: **Rectal temperature values for unfit (Phase 2) dogs during exercise. Values are expressed as mean ± sem of 4 treadmill runs per dog**

Running time (min)	Mean ± sem all dogs	Dogs above mean value	Dogs below mean value	Significance of differences between unfit dogs
Rest	38,1 ± 0,07	ABCDE	FGH	ns
10	38,8 ± 0,08	BDF	ACEGH	***
20	39,3 ± 0,11	BDF	ACEGH	***
30	39,3 ± 0,12	BEFG	ACH	***
40	39,2 ± 0,14	BEG	ACH	***
50	38,7 ± 0,20	B	AH	*
60	38,6 ± 0,12	AH		ns

Table 6: **Rectal temperature values for fit (Phase 4) and unfit (Phase 2) dogs during exercise. Values are expressed as the mean ± sem of 4 treadmill runs per dog**

Running time (min)	Unfit	Fit	Significance of difference fit : unfit
Rest	38,1 ± 0,08	37,6 ± 0,07	***
10	38,8 ± 0,06	38,5 ± 0,06	***
20	39,4 ± 0,21	38,5 ± 0,20	**
30	39,4 ± 0,09	39,3 ± 0,07	ns
40	39,4 ± 0,10	39,4 ± 0,07	ns
*50	39,2 ± 0,21	39,8 ± 0,08	**
*60	39,0 ± 0,30	39,8 ± 0,09	**

Dogs A and H only completed over 50 min running when unfit

Table 7: **Change from resting value in response to exercise of certain parameters[a] at Weeks 3 and 8 of training (Phase 3)**

Parameter	n	Mean ± 2SD increase above resting value Week 3	Week 8
CK (U ℓ^{-1})	8	50 ± 30	8 ± 3***
Lactate (μmol ℓ^{-1})	8	120 ± 20	73 ± 24*
Heart rate (beats min^{-1})	8		
10 min exercise		110 ± 14	89 ± 8*
30 min exercise		138 ± 16	90 ± 7*
60 min exercise		136 ± 17	15 ± 10*
5 min post exercise		33 ± 15	15 ± 7*

[a]Only parameters which showed significant differences in change from rest between Weeks 3 and 8 of training, are given

rising levels of heart rate and rectal temperature in relation to the values obtained when the dogs were fit.

ACKNOWLEDGEMENTS

Mrs M S Lombard, Miss V M Killeen, Mr J J Van Rensburg and Dr G E Swan are thanked for their assistance with this project. We would also like to thank the University of Pretoria for research funds provided, and the Electricity Supply Commission for the loan and feeding of the experimental animals.

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Table 8: **Mean change from resting value at Weeks 3,5 and 8 of detraining (Phase 5) for heart rate and rectal temperature**

Parameter	Confinement period (weeks)	Mean change from rest during exercise 10 min	during exercise 30 min	post exercise 5 min	Running time (min)
Heart rate (beats min^{-1})	3	126	132	57	60
	5	138	135	84	30
	8	140	-	80	20
Rectal temperature (°C)	3	0,4	0,6		60
	5	1,4	2,2		30
	8	1,4	-		20

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HORIZONTAL TRANSMISSION IN SHEEP AND DELAYED CLEARANCE IN GUINEA PIGS AND MICE OF A *BRUCELLA MELITENSIS* REV. 1 MUTANT

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ABSTRACT:
Virulence assays in guinea-pigs, mice and sheep with a Rev. 1 mutant strain (FSA) demonstrated that the latter had increased virulence in comparison with a reference strain. The mutant showed slower clearance in guinea-pigs and mice when compared with standard Rev. 1 strains and horizontal transmission was achieved in 2 sheep.

Key words: Rev 1, mutant, horizontal transmission

Hunter P.; Pefanis S.M.; Williamson C.C.; Botha W.J.S.; Van Schalkwyk M.S. Horizontal transmission in sheep and delayed clearance in guinea pigs and mice of a *Brucella melitensis* Rev. 1 mutant. *Journal of the South African Veterinary Association* (1989) 60 No. 2, 92-94 (En) Veterinary Research Institute, Onderstepoort, 0110 Republic of South Africa.

INTRODUCTION

The Rev. 1 mutant of *Brucella melitensis* has been used for many years in vaccines against *B. melitensis* in sheep and goats[11] and *B. ovis* in sheep[19]. In South Africa the vaccine is employed to protect rams against epididymitis caused by *B. ovis*. The efficacy of Rev. 1 in protecting against *B. ovis* has been vouched for[8 9 10 21] with the proviso that the rams are vaccinated at weaning.

Pieterson et al.[18] isolated a Rev. 1 strain (which they designated FSA) from aborting ewes and Angora goat rams suffering from orchitis, which had been vaccinated with Rev. 1. The Rev. 1 vaccine seed being used at the time, was compared and found to be identical to the FSA isolates. Biochemically the isolates strongly resembled a Rev. 1 strain but differed from the latter by achieving a colony size of 1,25 - 1,5 mm as well as showing no inhibition by basic fuchsin and thionin with aerobic incubation.

The FSA mutant was apparently selected when cloning a Rev. 1 strain for smooth colonies, the inclusion of which is essential for the production of an immunogenic vaccine. The FSA isolate seemed to have reverted to some of the characteristics of a wild *B. melitensis* strain and a concomitant increase in pathogenicity was expected.

This experiment was performed to determine whether the FSA mutant was more pathogenic than a standard Rev. 1 strain. Clearance of the organism was evaluated in mice and guinea pigs, and clearance and horizontal transmission were evaluated in sheep.

MATERIALS AND METHODS

Bacterial strains

Lyophilised cultures of Rev. 1 (FSA) were used for each experiment. The cultures were reconstituted with saline and diluted according to the infective dose required for the experiment. A Rev. 1 strain obtained from INRA (Drs Verger and Plommet, Institut National de la Recherche Agronomique, Nouzilly, France) was used as a reference strain in the mouse model.

Media

Brucella agar[6] was used for culture of all organ samples, milk and vaginal swabs. The plates were incubated aerobically at 37°C and were considered negative if no growth was observed within 7 d.

Processing of organs

Guinea-pig and mouse organs were homogenised and inoculated onto agar plates. Sheep organs were not homogenised but sections of the large organs were taken and the surface inoculated onto the agar plate. Lymph nodes were bisected and the full surface was smeared onto the surface of the agar. Milk samples were plated onto Brucella agar[6] using a sterile cotton swab.

Serology

Sheep sera were tested using the Serum Agglutination test (SAT)[13], Complement Fixation test (CFT)[12] and the Rose Bengal test (RBT)[3].

Sheep experiment

Pregnant (4 months gestation) Dorper ewes (n=5) known to be unvaccinated and negative on serology, were housed in isolation facilities, 4 x 1 m crate on a wire floor. Two of the ewes were inoculated subcutaneously with the FSA isolate, 2 - 4 x 10^9 live organisms per sheep, which is equivalent to a vaccine dose. The ewes were observed daily for signs of illness or abortion. When the ewes lambed, vaginal swabs were taken not later than 24 h after lambing and isolation of FSA was attempted. Vaginal swabs were again taken at 2 weeks post-lambing with the aid of a speculum. Milk samples were also collected aseptically from each ewe.

Serum was collected from the ewes and their lambs at 10, 30 and 40 d post-lambing. The ewes and their lambs were euthanased 3 months after initial infection. The organs were processed and cultured in an attempt to isolate FSA. The following organs were cultured: left and right sub-maxillary, parotid, retropharyngeal, prescapular, popliteal and iliac lymph nodes, spleen and udder tissue (from the ewes).

Guinea-pig test

Guinea-pigs (n=24) were injected subcutaneously with 10^3 organisms each of the FSA isolate. Eight guinea-pigs were slaughtered at intervals of 3, 5 and 6 months. Spleens were homogenised in 10 ml of Brucella broth[6], and 0,5 ml spread over the surface of a Brucella agar[6] plate (representing approximately 5% of the total spleen volume).

Mouse test

Mice (n=20) were each inoculated subcutaneously with 10^5 FSA organisms[4 5]. A second group of 20 mice were inoculated with 10^5 organisms each of a Rev. 1 strain from INRA. Ten mice of each group were killed at 30 d, and the rest 45 d post-infection.

The spleen of each mouse was homogenised in a small amount of saline and the entire spleen homogenate plated onto 2 Brucella agar plates. A positive result was recorded if one colony or more was found on the agar plate.

RESULTS

Sheep experiment

All 5 ewes remained healthy and lambed normally, giving birth to viable lambs, one month after 2 of the ewes had been inoculated with the FSA isolate.

Results of the culture of the vaginal swabs and milk samples are recorded in Table 1. Table 2 records the results of serology on the ewes and lambs. Post-lambing vaginal swabs yielded positive cultures of *B. melitensis* (FSA) in one ewe (No. 5). At 14 d all the vaginal swabs were negative but a milk sample from an uninfected ewe (No. 4) yielded a positive culture of FSA. The ewe which yielded the positive vaginal swab became seropositive 30 d post-lambing. The same ewe was seronegative 10 d post-lambing. Ewe No. 4 which yielded a positive milk sample did not seroconvert. Table 3 illustrates the sequence of lambing of the ewes. Tissue samples taken from the ewes and lambs 3 months after initial infection of the 2 ewes, were negative on culture for FSA. None of the lambs became seropositive.

Guinea-pig test

Cultures of the guinea-pig spleens were positive for *B. melitensis* FSA up to 6 months after infection. At 3 months 100% (8/8) were infected, at 5 months 50% (4/8) and at 6 months 37,5% (3/8) were still infected.

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Received: January 1988 Accepted: January 1989

Mouse test

At 30 d the group infected with the INRA Rev. I strain yielded 80% spleens positive on isolation while at 45 d 60% were positive. The FSA group showed a rise from 60% positive at 30 d to 90% infected at 45 d.

DISCUSSION

Various characteristics of the FSA variant indicate that the strain is more virulent than standard Rev. I strains: 1. Its bichemical characteristics as described by Pieterson *et al.*,[18] indicate a similarity with a virulent *B. melitensis* strain[6]; 2. After many years of use of Rev. I vaccines in other countries, only one case of epididymitis has been reported[15], while the FSA isolate caused a high percentage of epididymitis in Angora goat rams[18]; 3. The results of the guinea-pig and mouse tests indicate slower clearance than is usual with Rev. I strains. The mouse model used by Bosseray[5] to evaluate the persistence of different Rev. I strains in mouse tissues, indicated that there is considerable reduction of organisms in mouse spleens by Day 45 after infection. The results in this study are similar to those achieved in a study with a virulent strain[4] of *B. melitensis* where infection of the spleen remained on a plateau until 10 weeks, after which clearance began to take place. In guinea-pigs inoculated with 10^3 organisms of classic strains of Rev. I, infection is cleared from the spleen by 12 weeks post-inoculation[14 16]. The guinea-pigs infected in this study remained infected up to 6 months later, at the same infective dose; 4. The apparent ability of the FSA isolate to be transmitted horizontally to 2 susceptible ewes, one of which seroconverted, is supportive evidence for the increased virulence of the variant. The sequence of lambing confirmed that transmission of FSA could have taken place. This is the first record of horizontal transmission of a Rev. I strain. Previous attempts to transmit Rev. I strains horizontally in sheep have failed[17] as well as attempts to produce an increase in virulence of Rev. I by passage through pregnant goats[1-7].

It appears that the FSA variant was selected by cloning of smooth colonies for vaccine seed production. It was no doubt chosen for its relatively vigorous growth characteristics as well as its smooth state. In vitro selection of smooth clones has been shown to produce isolates with a higher infectivity index[20] than rough phase colonies. However, other workers[2] state that after 7 years' deliberate search, they were unable to produce strains of increased pathogenicity.

Table 1: **Results of culture of vaginal swabs and milk samples taken from ewes exposed to *B. melitensis* FSA**

Sheep no.	Vaginal swabs post lambing		Milk samples
	24 h	14 d	14 d
1*	-	-	
2*	+	-	-
3	-	-	-
4	-	-	+
5	+	-	-

* ewes inoculated with *B. melitensis* (FSA)
\+ positive culture of *B. melitensis* (FSA)

Table 1: **Post-lambing serology performed on ewes exposed to *B. melitensis* FSA**

	10 d			30 d			40 d		
Sheep no.	RBT	SAT IU ml^{-1}	CFT IU ml^{-1}	RBT	SAT IU ml^{-1}	CFT IU ml^{-1}	RBT	SAT IU ml^{-1}	CFT IU ml^{-1}
1*	P	84	24	P	84	-	P	19	30
2*	P	84	18	P	84	98	P	54	30
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	P	74	98	-	-	-
All lambs	-	-	-	-	-	-	-	-	-

* ewes inoculated with *B. melitensis (FSA)*
P = positive

Table 3: **Sequence of lambing of the 5 ewes exposed to Rev. I FSA**

Sequence of lambing	No. of ewe	Experimentally infected	Isolation	Serology
1	1	yes	-	+
(2 d interval)				
2	3	no	-	-
(5 d interval)				
3	2	yes	+ vaginal	+
(1 d interval)				
4	4	no	+ milk	-
(4 d interval)				
5	5	no	+ vaginal	+

Failure of the isolate to be transmitted vertically to the lambs of the affected ewes, to be cleared from the tissues of the infected sheep by 3 months and to retain some of the biochemical characteristics of a typical Rev. 1 isolate, indicate that the FSA variant does not have the full virulence of a wild *B. melitensis* strain.

The experiences of the authors with the FSA variant strain of *B. melitensis* Rev. 1, highlight the importance of monitoring the relevant biochemical characteristics of seed material for vaccine production.

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THE SEASONAL TICK POPULATIONS ON TRADITIONAL AND COMMERCIAL CATTLE GRAZED AT FOUR ALTITUDES IN NATAL

MAUREEN K BAKER*, F B W DUCASSE**, R W SUTHERST*** and G F MAYWALD***

ABSTRACT

The number of ticks collected over a period of one year from cattle at 4 altitude levels in Natal, Republic of South Africa, are plotted against meteorological and seasonal data. Collections were made from both traditional and commercial cattle at each altitude. Most of the economically important tick species show clear seasonal patterns of activity. This suggests that strategic dipping could be used as a means of control.

Key words: Ticks, collection, altitude, Natal, meteorological, seasonal, cattle, strategic, dipping

Baker M.K.; Ducasse F.B.W.; Sutherst R.W.; Maywald G.F. The seasonal tick populations on traditional and commercial cattle grazed at four altitudes in Natal. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 95-101 (En.) 65 van Heerden Street, 0084 Capital Park, Republic of South Africa.**

INTRODUCTION

The variations in the size and seasonal patterns of tick populations on cattle determine the most effective control strategies. Climate, as well as animal breed and management, are known to have powerful effects on tick populations[2 3 12]. There have been some studies on the population dynamics of ticks on cattle in the eastern Cape Province of South Africa by Norval[4] and Rechav[9 10]. Baker & Ducasse[1] were, however, the first to study the population dynamics of ticks in southern Africa, when they collected data from cattle at 9 sites in central Natal at 4 altitudes ranging from sea level to above 1 500m. The cattle were selected from both a traditional and a commercial herd at each altitude. These regular collections allowed comparisons to be made between the species, composition and seasonal patterns of tick numbers in each farming system at each altitude. This paper presents the seasonal data collected during 1966-67. These data were used to tune the simulation model of Floyd, Sutherst & Maywald (in prep.) describing the life cycles of the 3-host tick species *Rhipicephalus appendiculatus* and *Amblyomma hebraeum*.

METHODS

Baker & Ducasse[1] described the tick collection procedures in detail. The locations of the collection sites are shown in Fig. 1. They were chosen to be representative of farms at each of 4 altitude levels in Natal. The salient features of each collection site are listed in Table 1.

RESULTS

The numbers of each stage of the commonest species collected on each farm are shown in Fig. 2-10. There were marked changes in the composition of species at different altitudes, the intermediate zone being the most suitable for the economically important ticks.

Boophilus microplus was mainly confined to the coastal belt, but was also present, together with *Boophilus decoloratus*, on the farm belonging to Hampson at 300m above sea level. Further inland, only *B. decoloratus* was encountered. *R. appendiculatus* occurred at all but the highest altitudes and was usually more numerous on commercial farms. *Rhipicephalus evertsi evertsi* was found in the same areas as *R. appendiculatus* but there was no apparent preference for commercial

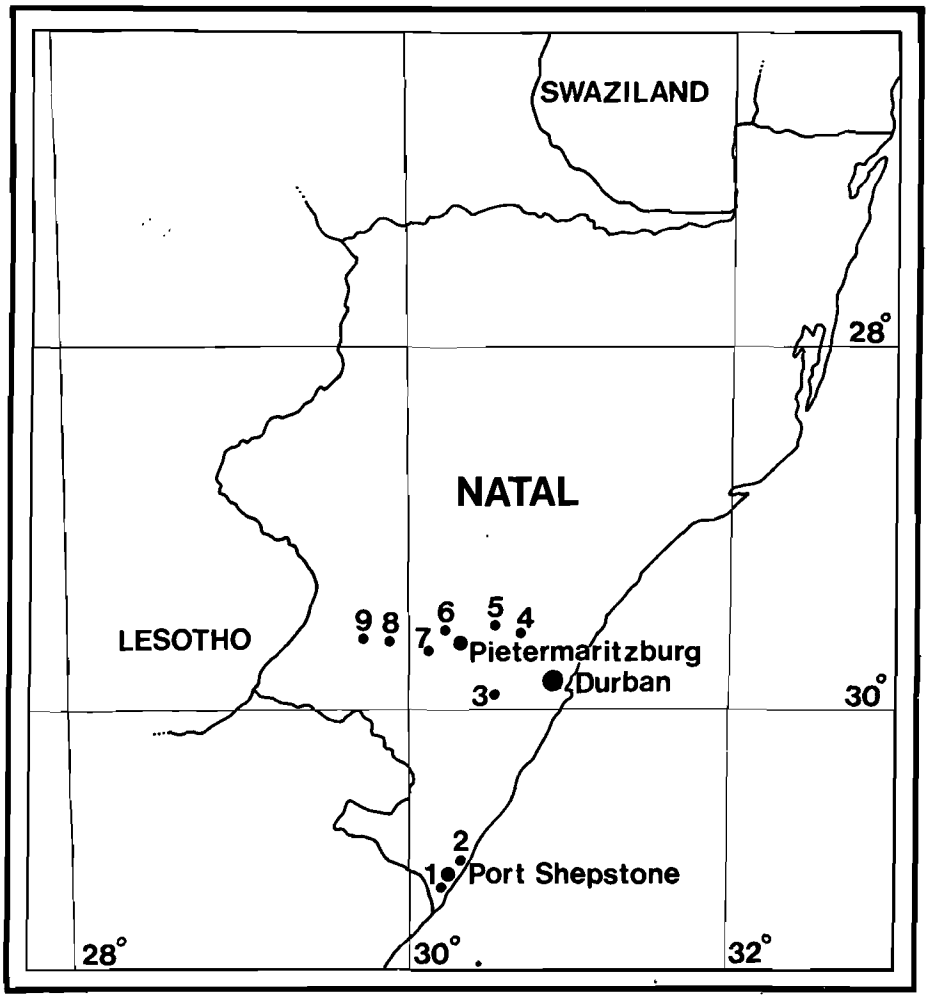

Fig. 1: **Map of Natal showing the geographical location of each collection site (For details of each site see Table 1).**

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Received: January 1989 Accepted: February 1989

Table 1: **Summary of the geographical locations of the farms in Natal on which collections were made**

CODE	FARM	ALTITUDE	COMMENTS
	(a) **Coastal**	**(0-300m)**	
1*	Sommerville	0	Lush coastal bush and grassland
2	Tank area 85	0	Tribal area, overgrazed with some bush
	(b) **Thornveld**	**300-900m**	
3*	Hampson	300	Sugar cane, good soil, bush
4	Tank area 224	600-750	Steep slopes in Valley of a Thousand Hills, overgrazed, degraded pasture
5*	Blore	600-900	Friesians, poor tick control
	(c) **Mistbelt**	**900-1500m**	
6*	Carr	1100	Adjacent to Midmar Dam, irrigated mixed pastures
7*	Tank area 56	1300	Taylor's Halt: steep, treeless, degraded area with erosion
	(d) **Highveld**	**1500+m**	
8	Tank area 118	1500-1800	Bleak, treeless sourveld
9*	Campbell	1500-1800	

* Meteorological data available

farms. *Rhipicephalus simus* also had a wide tolerance of climatic conditions but throughout the study it was found to be an uncommon tick. *A. hebraeum* was found only in the thornveld zone. *A. hebraeum, Hyalomma marginatum rufipes, Haemaphysalis silacea* and *Ixodes pilosus* were all sporadically distributed at the lower altitudes, usually on commercial farms. *Rhipicephalus lunulatus, Rhipicephalus follis, Ixodes drankensbergensis, Margaropus winthemi* and *Otobius megnini* were rare and confined mostly to traditional farming areas at the higher altitudes.

The total number of adult female ticks per animal per year at each location is given in Table 2.

A summary of the seasonal incidence of the various tick species is given in Table 3. Autumn and spring are the seasons with greatest tick activity.

DISCUSSION

The results demonstrate the variations in tick populations at different altitudes but at the same latitude in a subtropical environment in southern Africa. As such, they provide an ideal medium with which to interpret the climatic preference of different tick species, as well as their population dynamics. The incidence of all the common species declined greatly at the highest altitude.

It can be seen from Table 2 that the numbers of *B. microplus* at coastal levels are considerable. This is also true of *B. decoloratus* on the thornveld farm belonging to Blore, and in the mistbelt regions. The numbers of *Boophilus* spp. encountered at Hampson and T/A 224 are comparatively lower. The senior author (Baker, unpublished data) has found *B. microplus* on cattle at a Tank area adjacent to T/A 224 in the Valley of a Thousand Hills. It can be speculated that the reduced numbers of *B. microplus* and *B. decoloratus* at this altitude reflect a battle between the species, with possible sterile hybridisation leading to a natural reduction in numbers as found by Norval & Short[6] and Sutherst[15]. The incidence of *B. decoloratus* in Natal is greatly in excess of that found by Matson & Norval[3] in Zimbabwe. The seasonal activity of both *B. decoloratus* and *B. microplus* peaks during spring and autumn. As in Zimbabwe[5], ticks were encountered throughout the year, albeit in low numbers at times. It was only at altitudes above 1500m that no ticks were encountered during the midsummer period.

R. appendiculatus and *R. evertsi evertsi* were present in appreciable numbers at all altitudes below 1500m. Above 1 500m the terrain and vegetation were unsuited to *R. appendiculatus*[8 13 14]. *R. appendiculatus* was more numerous on commercial farms, possibly due to the presence of more scrub and bush for the protection of the immatures. Traditional areas are in general stripped of bush and overgrazed[7]. The predominance of zebu type cattle in the traditional areas would also influence the situation because they are more resistant to tick infestation[2 11 12]. The seasonal pattern of *R. appendiculatus* was that larvae were most active in autumn, nymphae during autumn and spring, whilst the adults were most active during the summer months. These findings were later supported by those made in Zimbabwe[7 13]. Both *R. evertsi evertsi* immatures and adults were active throughout the year, but the adults tended to show some increase in activity during the summer and autumn, as in Zimbabwe[5]. The numbers of *R. simus* found in the Natal survey were consistently low and adult activity took place mainly from spring to autumn, although ticks were found throughout the year. Only adult *R. lunulatus* and *R. follis* were collected and then mainly during the spring and autumn months.

Localised conditions clearly favoured some species, such as *A. hebraeum* in the thornveld area of T/A 224 and on the Hampson property. *A. hebraeum* was only found in meaningful numbers at T/A 224, where larvae and nymphae were present throughout the year, with small peaks in autumn and winter respectively, suggesting that the micro-climate in the sheltered gullies and valleys in this area were optimal for this species. The adults, however, show a definite increase in activity in spring and summer. The findings in Natal, indicate that *A. hebraeum* may complete its life cycle within the year. The studies by Rechav[8 9] in the eastern Cape Province of S.A. corroborate the Natal findings.

H. marginatum rufipes was never present in any great quantity and most adults were collected during the summer months. *H. silacea* and *I. pilosus* were only encountered on the Hampson property, where they were active throughout the year. Their presence could be attributed

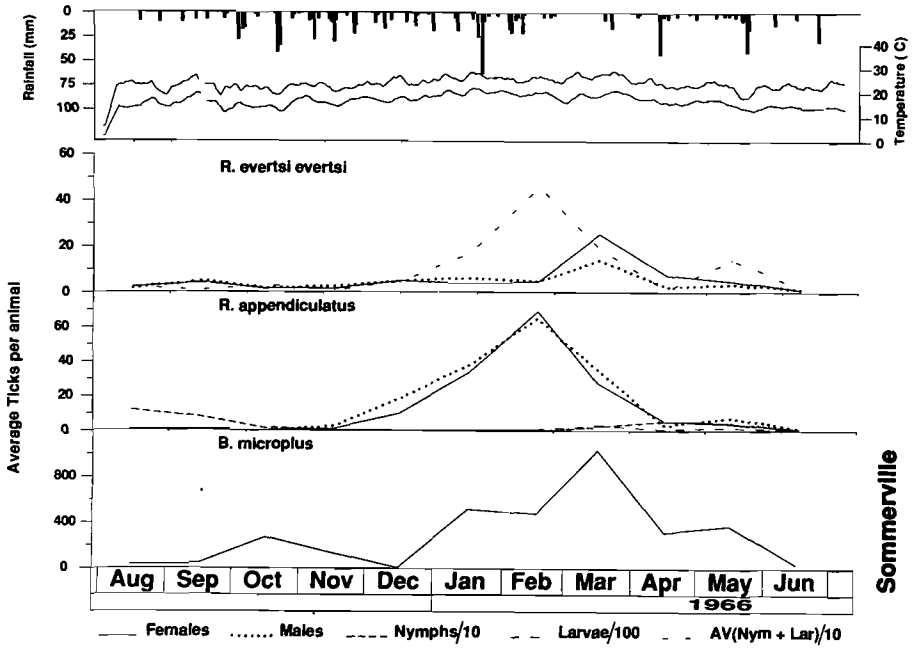

Fig. 2: **Sommerville - the meteorological data, mean number of ticks collected and seasonal variation of each species**

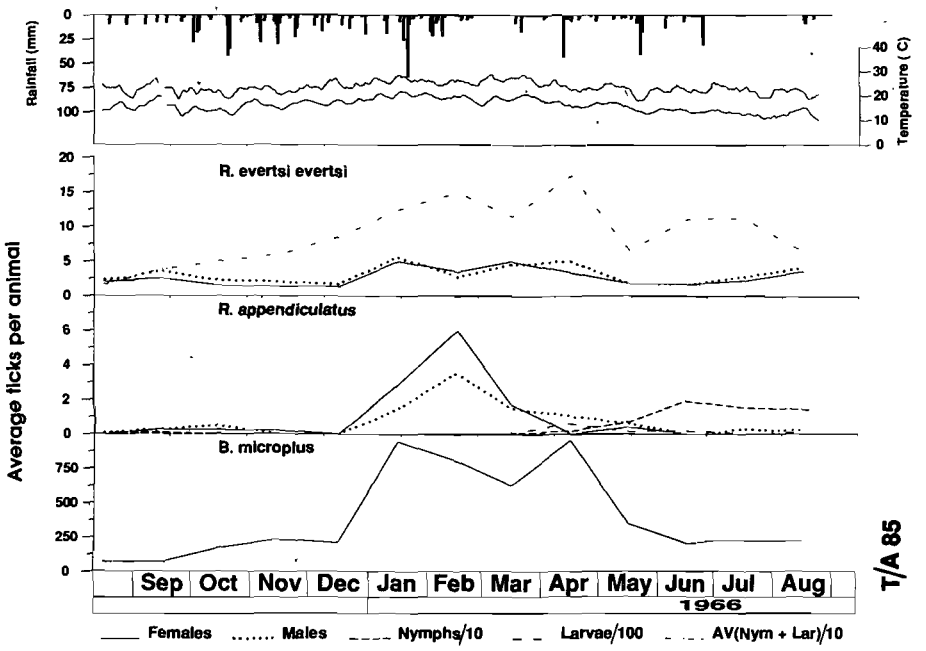

Fig. 3: **Tank area 85 - the meteorological data, mean number of ticks collected and seasonal variation of each species**

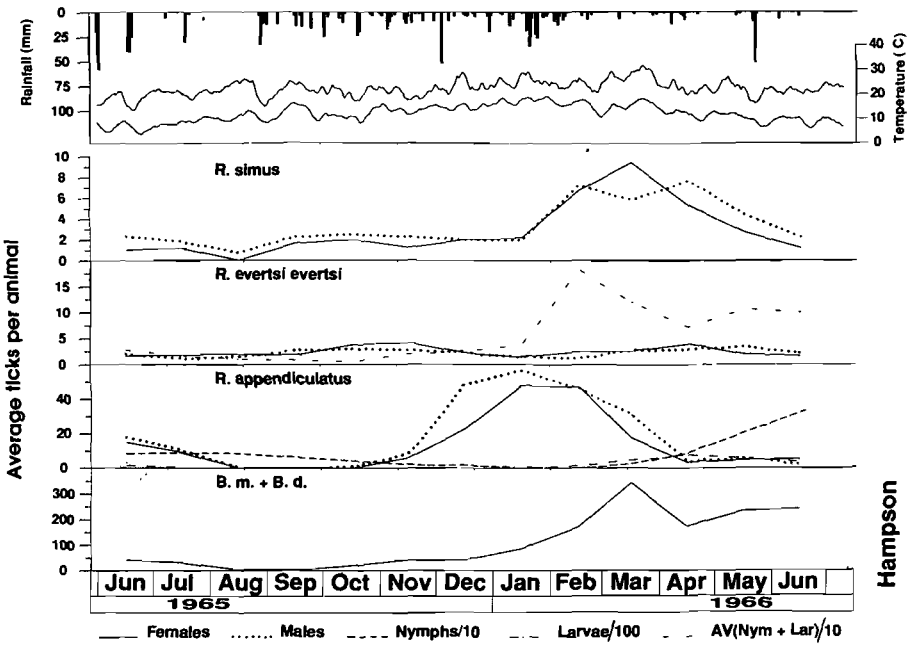

Fig. 4a: **Hampson - the meteorological data, mean number of ticks collected and seasonal variation of each species. (B.m. =** *B. microplus* **& B.d. =** *B. decoloratus*).

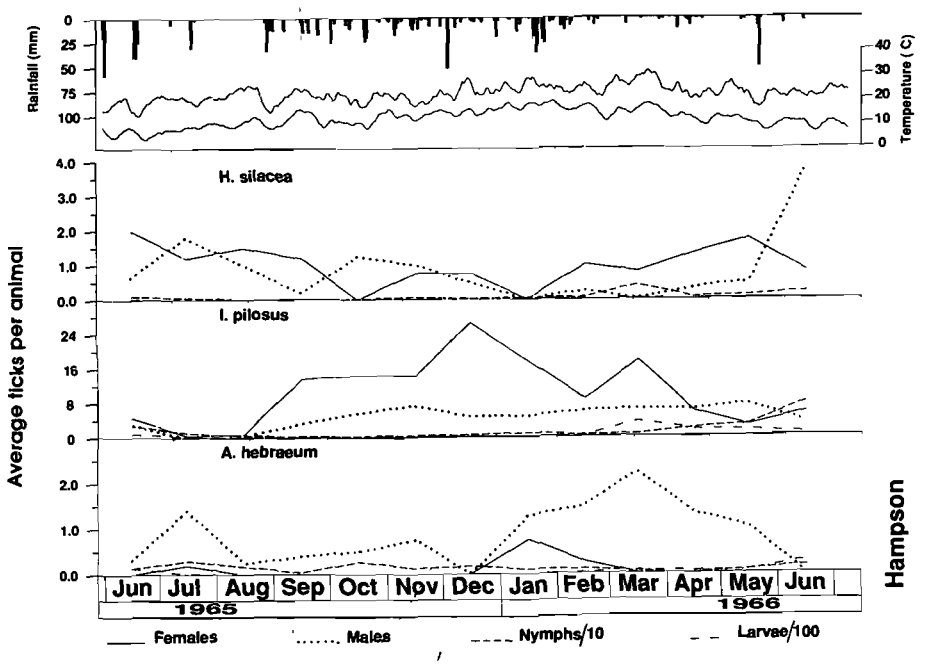

Fig. 4b: **Hampson - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

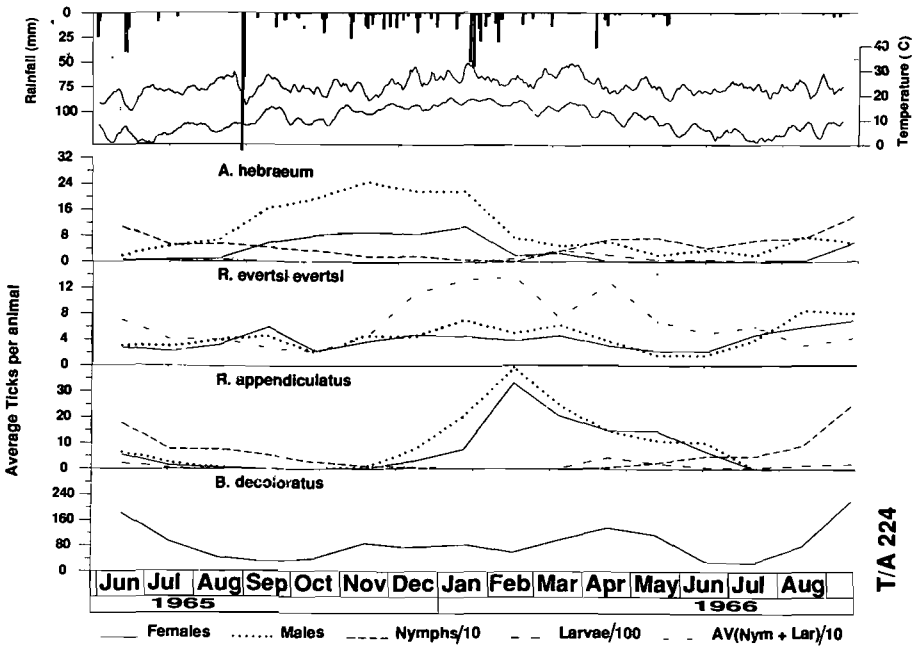

Fig. 5: **Tank area 224 - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

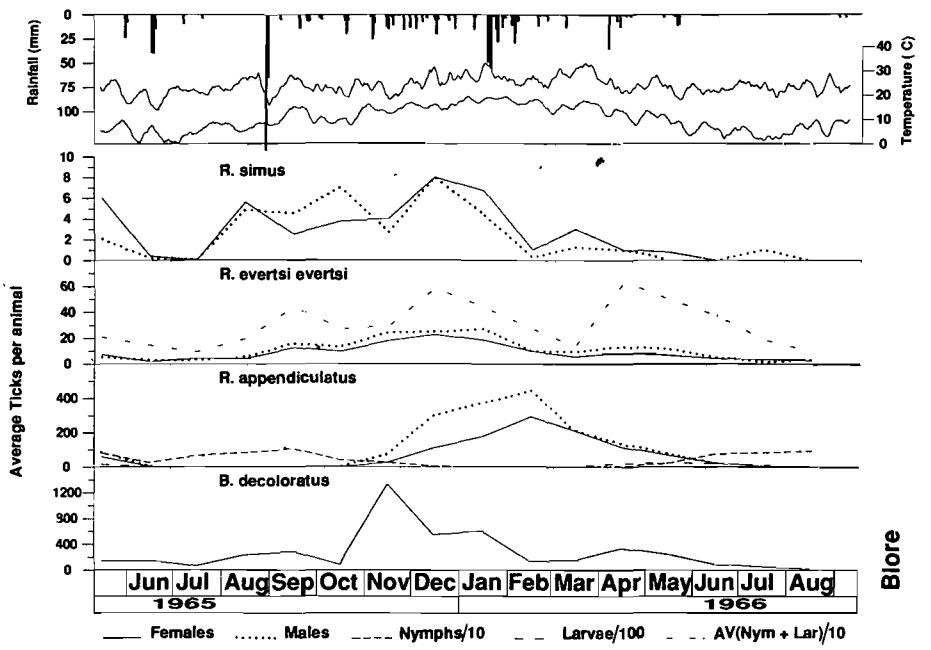

Fig. 6: **Biore - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

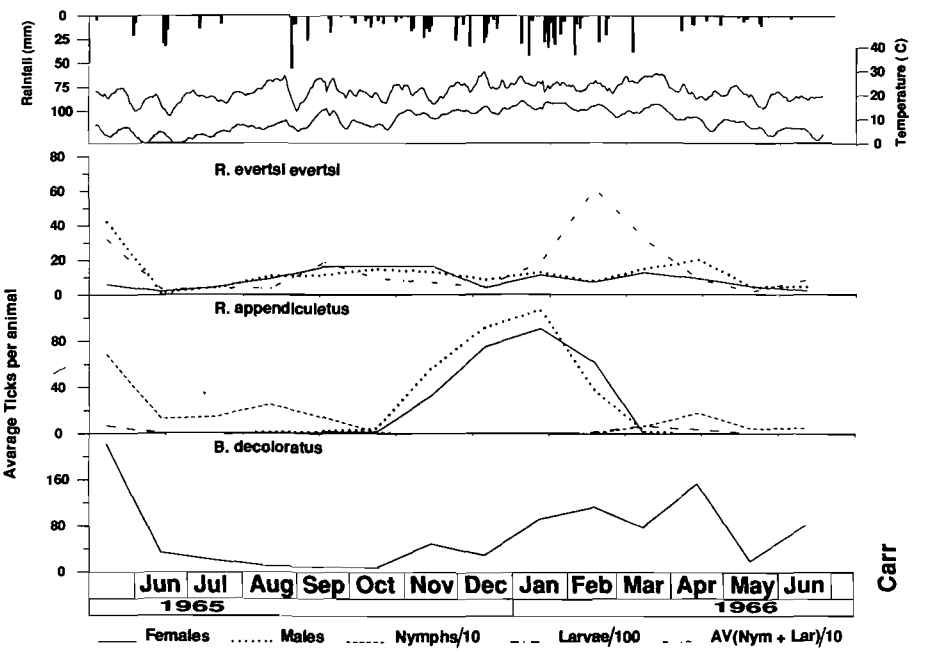

Fig. 7: **Carr - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

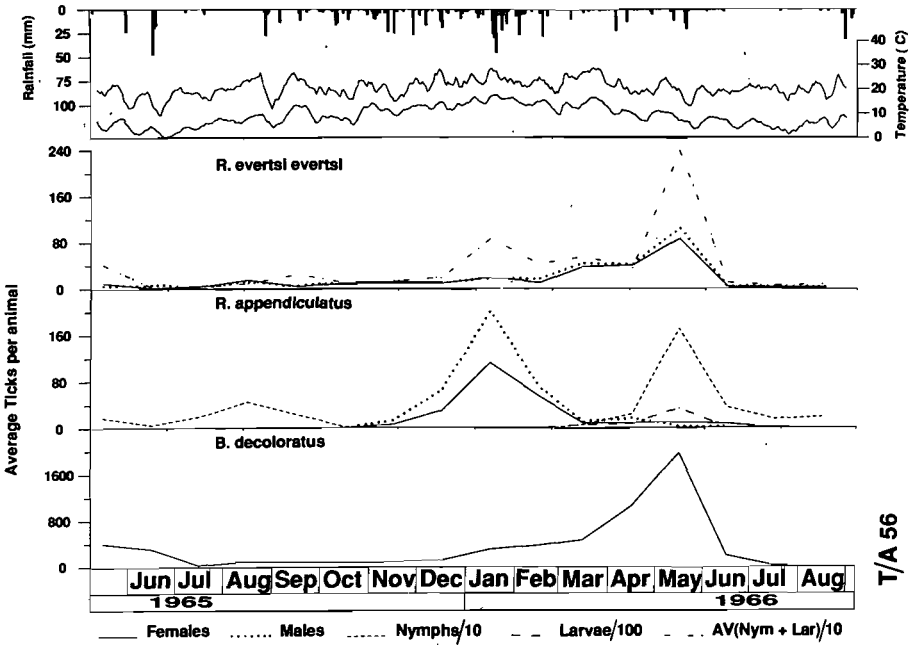

Fig. 8: **Tank area 56 - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

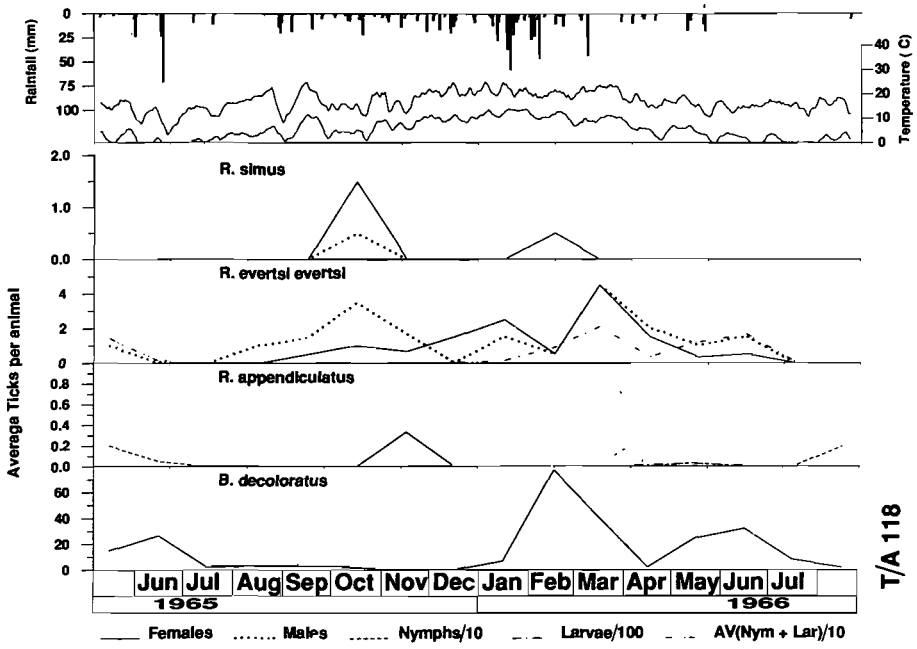

Fig. 9a: **Tank area 118 - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

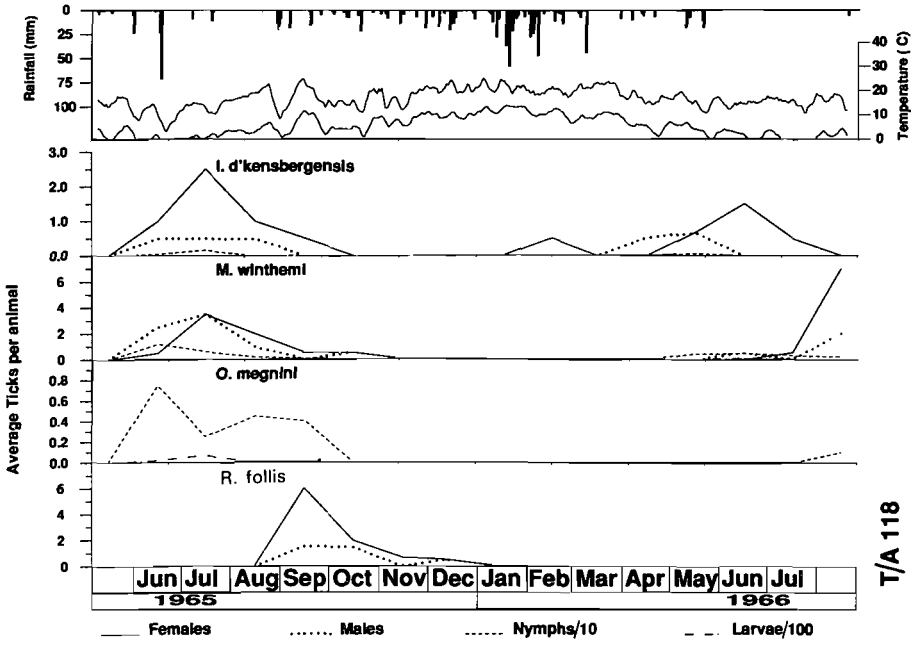

Fig. 9b: **Tank area 118 - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

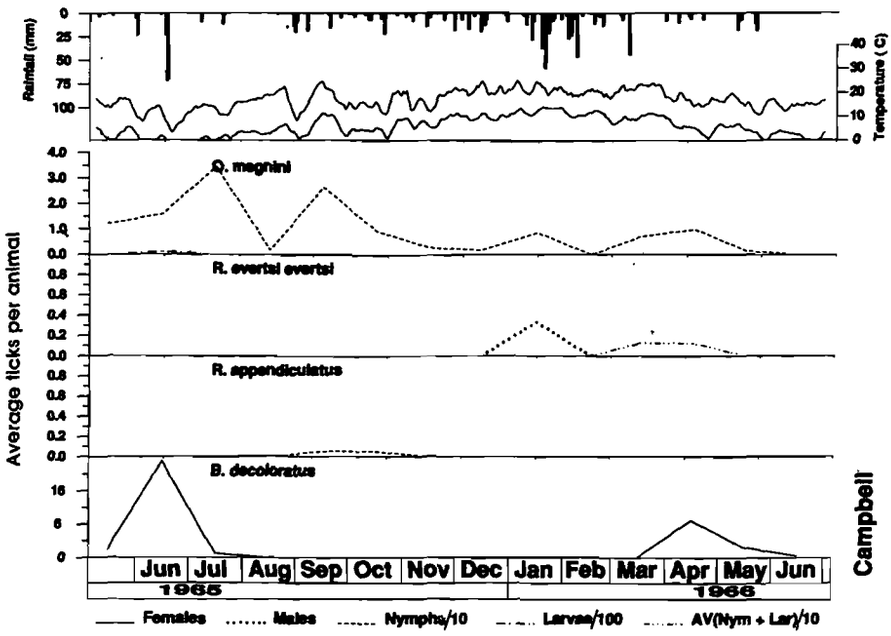

Fig. 10. **Campbell - the meteorological data, mean number of ticks collected and seasonal variation of each species.**

to the presence of natural bush and small antelope and other game animals.

Thus, the seasonal incidence of most tick species was similar, with the adults feeding in spring and summer. Only *I. drakensbergensis, M. winthemi* and *O. megnini* were more active in the cooler months. These results suggest that dipping in spring, summer and extending into autumn will control most of the common species.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Jane Walker for her valuable assistance in preparing the manuscript.

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Table 2: **The total number of adult female ticks per animal per year at each location.**

	Sommerville	T/A 85	Hampson	T/A 224	Biore	Carr	T/A 56	T/A 118	Campbell
Boophilus microplus	14195	20706	-	-	-	-	-	-	-
Boophilus decoloratus	-	-	-	4914	15113	3006	15246	501	51
Boophilus ssp	-	-	6108	-	-	-	-	-	-
Rhipicephalus appendiculatus	623	50	729	444	3843	1012	808	1	-
Rhipicephalus evertsi evertsi	267	141	129	240	478	489	817	28	-
Rhipicephalus simus	123	-	154	-	139	6	7	4	-
Rhipicephalus lunulatus	-	-	-	-	-	-	34	-	-
Rhipicephalus follis	-	-	-	-	-	-	-	19	-
Hyalomma marginatum rufipes	-	-	-	-	40	-	-	-	-
Haemaphysalis silacea	-	-	53	-	-	-	-	-	-
Amblyomma hebraeum	-	-	5	213	-	-	-	-	-
Ixodes pilosus	-	-	558	-	-	-	-	-	-
Ixodes drankensbergensis	-	-	-	-	-	-	-	17	-
Margaropus winthemi	-	-	-	-	-	-	18	22	0

Table 3: **Summary of the seasonal incidence**

	LARVAE	NYMPHS	ADULTS
Boophilus decoloratus	Spring/Autumn	Spring/Autumn	Spring/Autumn
Boophilus microplus	Spring/Autumn	Spring/Autumn	Spring/Autumn
Rhipicephalus appendiculatus	Autumn	Autumn, Spring	Summer
Rhipicephalus evertsi evertsi	All year	All year	Summer/Autumn
Rhipicephalus simus			Spring/Summer
Rhipicephalus lunulatus			Spring/Autumn
Rhipicephalus follis			Spring
Hyalomma marginatum rufipes			Summer
Haemaphysalis silacea	All stages *any time*		
Amblyomma hebraeum	Autumn	Winter	Spring/Summer
Ixodes pilosus	Autumn/Spring	Spring/Autumn	Spring/Autumn
Ixodes drakensbergensis	*Winter/Spring*	Winter/Spring	Winter/Spring
Otobius megnini	Immatures Autumn/Spring		
Margaropus winthemi	Autumn/Spring	Autumn/Spring	Autumn/Spring

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Book review/Boekresensie

DERIVED INTERVENTION LEVELS FOR RADIONUCLIDES IN FOOD GUIDELINES FOR APPLICATION AFTER WIDESPREAD RADIOACTIVE CONTAMINATION RESULTING FROM A MAJOR NUCLEAR ACCIDENT

World Health Organisation, Geneva, 1988 pp60. Price Sw 11 (ISBN 92-4-154233-0). Available in RSA from Van Schaiks Bookstore (Pty) Ltd., P.O. Box 724, 0001 Pretoria.

Following the major nuclear accident that occurred in Chernobyl, USSR, in 1986, it became clear that action was needed for dealing with widespread radioactive contamination that affected many countries at considerable distances from the accident site.

This publication was prepared from reports of 2 WHO meetings which were held in 1987 on the subject and is concerned mainly with the use of food that may be contaminated in the "far field" (the area far removed from the accident site) and does not include guidance on the actions needed close to the accident site. The publishers give the following information concerning this book:

"This publication meets the need for guidance in managing the public health problems posed by widespread contamination of food and drinking water following a major nuclear accident. Intended to help national authorities introduce appropriate protective measures, the book presents guidelines for the calculation and application of derived intervention levels below which action to reduce or avoid the potential health detriment would not be justified".

This publication is very technical: it describes in detail the methods used to calculate the criteria for both individual dose and population dose levels for intervention, including cost-benefit analysis. This book is recommended for authorities involved in policy making as regards a disaster of such a nature and will be of limited interest to the veterinarian directly involved in public health work.

D N LLOYD

THE INFLUENCE OF BREED AND SEX ON THE INCIDENCE OF MORTALITIES AND SKIN TEARS IN BROILER CARCASSES

N H CASEY*, G A SMITH and R I CROSLEY

ABSTRACT

The effects of nutrition, breed and sex on the incidence of mortalities and of skin tears on broiler carcasses were studied. Both sexes of 2 breeds, Hubbard and Ross, were given 4 dietary treatments consisting of a normal level of vitamin and mineral premix plus 300 ppm furazolidone, twice normal premix inclusion level plus 300 ppm furazolidone, a normal level of vitamin and mineral premix, and twice normal mineral premix inclusion level. A coccidiostat containing the active ingredient halofuginone was included at 6 ppm, twice the recommended dosage. Starter, grower and finisher diets were fed respectively up to Day 20, Day 30 and Day 48. Group growth performances, mortalities and the incidence of torn skin were monitored on both the live birds and carcasses. The effect of the different nutritional treatments on skin tears was not significant (P=0,9533), as was the breed effect (P=0,0547). However, the effect of sex was significant (P=0,0044), the incidence in hens being higher. Mortalities among the Hubbard were significantly greater than among the Ross (P=0,0001). Hens showed a slightly higher mortality rate than roosters, tending towards significance at the 5% level (P=0,0554).

Key words: Broilers, breed, sex, skin tears

Casey N.H.; Smith G.A.; Crosley R.I. The influence of breed and sex on the incidence of mortalities and skin tears in broiler carcasses. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 102-103 (En) Department of Livestock Science, Faculty of Agriculture, University of Pretoria, 0002 Pretoria, Republic of South Africa.**

INTRODUCTION

The incidence of skin tears in broiler carcasses was first noticed and reported by certain companies in the South African broiler industry during 1986 as water pockets under the skin of freshly slaughtered carcasses. Upon examination these pockets were found to contain a "gel" caused by water aggregation in the subcutaneous areolar tissue. The water had apparently entered the subcutaneous region from both minute and large tears in the skin. Further reports on the occurrence of torn skin were received from the industry together with speculations about the causes of the tears. These speculations included the effect of broiler strains, sex, mineral and vitamin imbalances and the presence of a coccidiostat containing the active ingredient halofuginone. A greater incidence of skin tears in carcasses of White Rock broiler hens treated with halofuginone compared with monensin has been reported[1]. This trial consequently tested possible nutritional, breed and sex effects which might induce skin tears.

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Received: June 1988 Accepted: January 1989

MATERIALS AND METHODS

Both sexes of 2 broiler strains (Hubbard and Ross) were divided into single-sex groups of 120 birds each. Four different treatments were fed (Table 1), using 4 replications per treatment. This gave a total of 7 680 birds divided into 64 groups. The birds were kept on the floor in 5m^2 pens (density 24 broilers/m^2) separated by wire mesh in a controlled environment house. Feed and water were available *ad lib* and the highest light intensity was used in a 23-hour photoperiod throughout the trial. The vitamin and mineral inclusion levels shown in Table 1 are supplied to the industry commercially and exceed the NRC recommendations[2]. All treatment groups were reared for 48 d, having received starter diets up to Day 20, grower diets to Day 30 and finisher diets thereafter, according to treatments listed in Table 1.

Halofuginone was included at 6 ppm in the starter and grower rations (recommended level 3 ppm) and at 3 ppm in the finisher ration in an attemp to increase the occurrence of skin tears. Group mass of each replication was measured on Days 1, 21, 31, and 48. Mortalities were recorded daily. The birds were examined for skin tears on Days 21, 31 and 48. All groups were wingbanded between Days 31 and 33. House temperatures were monitored daily, and they ranged between 25 and 28°C. On 2 occasions (Days 38 and 41) the temperature rose to 32°C when the micromist cooling system failed. On Day 48, 2 pens containing Ross roosters receiving Treatment 2, were flooded during the night. The 51 mortalities encountered in these pens were not included in the accumulated count of mortalities. No medication was given other than that stipulated in the treatments.

Slaughtering was done at a commercial abattoir which uses the same procedures as the abattoir at which the skin tears were first noticed, processing at a rate of 36 birds per min. Evisceration was done by hand. An observation point was established after evisceration at a bend in the line between the first and second spraywashes. All the carcasses were examined for tears, care being taken not to confuse poor evisceration procedure with skin tears. The numbers on the wing bands of all the carcasses showing torn skin were recorded.

The results were analysed in an analysis of variance procedure employing a Duncan's multiple range test for specific differences at $P<0,05$.

RESULTS

Of the original 7 680 day-old broilers, 430 Hubbard (275 roosters and 155 hens) and 220 Ross (125 roosters and 95 hens), (a total of 650 or 8,5%) died before Day 48, excluding the 51 lost due to flooding. Of the remaining 6 979 birds, 946 (13,6%) yielded carcasses with torn skin.

Skin tears

Torn skins were noticed from Day 25 onwards. Attention was drawn to these lesions by the appearance of blood on the feathers, yet there may have been unnoticed cases. All these tears occurred on the backs and rumps of the birds and were typically L-shaped as if a sharp hook had caught and torn the skin. Dietary treatments had no effect on the occurrence of skin tears (P = 0,9533). The effect of sex was highly significant (P = 0,0044). Of the number slaughtered, roosters and hens of the Ross strain recorded means of 3,0% and 7,1% respectively and the Hubbards 5,7% and 11,4%. The breed effect was not significant, although there was a tendency towards significance (P=0,0547). Of the total number of 946 carcasses with torn skin, 61,5% were Hubbard and 38,5% were Ross.

Mortalities

The 8,5% mortalities could be ascribed to many different causes of which no detailed records were kept, because the high density caused severe trampling once a bird had gone down. Collection of mortalities was done every morning. Neither dietary treatment nor sex had any significant effect on mortalities. Breed had a highly significant effect (P=0,0001) with the Hubbard recording 66,1% and the Ross 33,8% of the mortalities.

Table 1: **Dietary treatments given to groups of experimental broiler strains**

Dietary components (kg/t)	Starter	Grower	Finisher	Treatments 1	2	3	4
Yellow maize	460	609	640				
Fish meal	150	120	100				
Grain sorghum	100	100	100				
Sunflower oilcake	--	--	40				
Wheatenbran	100	17	--				
Soya oilcake	80	77	40				
Poultry by-product	50	50	50				
Hominy chop	37	--	--				
Feed lime	20	20	20,7				
Methionine	1,1	0,65	0,25				
Lysine	0,15	0,15	--				
Monocalsium phosphate	0,18	4,17	6,18				
Salt	--	0,32	0,99				
Halofuginone	1 kg (6ppm)	1 kg (6ppm)	0,5 kg (3ppm)				
Vit-min*				1 x	2 x	1 x	2 x
Furazolidone				300 ppm	300 ppm	--	--

Vit-min*

Starter (per ton) : Vit A (13 000 000 IU), Vit D (3 000 000 IU), Vit E (30 000 IU), Vit K (2 000 mg), Vit B (2 000 mg) Vit B_2 (6 000 mg), Vit B_6 (2 500 mg) Vit B_{12} (15 mg) folic acid (1 000 mg), biotin (100 mg), pantothenic acid (8 000 mg), niacin (30 000 mg), antioxidant (200 g), cholin chloride (active) (300 g), copper (15 g), manganese (70 g), zinc (40 g), cobalt (0,5 g), iodine (1 g,) iron (25 g), selenium (0,15 g)

Finisher (per ton) : Vit A (12 000 000 IU), Vit D (3 000 000 IU), Vit E (40 000 IU), Vit K (2 000 mg), Vit B (1 000 mg), Vit B_2 (5 000 mg), Vit B_6 (1 000 mg), Vit B_{12} (10 mg), folic acid (500 mg), biotin (50 mg), pantothenic acid (5 000 mg), niacin (20 000 mg), antioxidant (200 g), choline chloride (active) (300 g), copper (10 g), manganese (100 g), zinc (60 g), cobalt (0,5 g), iodine (1 g), iron (20 g), selenium (0,15 g), carophyll red (10 g)

Mass increase

Dietary treatments had no significant effect (P=0,0757). A highly significant sex effect was recorded, the males gaining faster than the females (P=0,0001). Breed had no significant effect (P=0,8487).

DISCUSSION

The non-significant treatment effect (P=0,95) on skin tears means that neither furazolidone nor the doubling of the mineral and vitamin inclusion levels can be held responsible, according to these results. The industry has reported an incidence of 25% at times, although this figure has not been verified. The incidence of 13,5% reported here is substantially lower than some of the claims from the industry. Nevertheless 13,5% remains alarmingly high. Virtually all the carcasses having torn skins would have had to be "downgraded".

Statistically there was no breed effect (P=0,0547) regarding the occurrence of skin tears, but there remains a tendency towards significance. Of the incidences of torn skin, 61,5% were from the Hubbard strain and 38,5% from the Ross. Of these percentages, Hubbard hens accounted for 41,0% and roosters for 20,5%, while Ross hens and roosters accounted for 27,2% and 11,3% respectively.

The conclusion drawn from this project is that the incidence of skin tears on broiler carcasses is sex related with a tendency towards a contributing influence by the breed.

ACKNOWLEDGEMENT

The authors wish to acknowledge with appreciation Roussel laboratories (SA) Pty Ltd for their financial support of the project, the Eastern Transvaal Agricultural Co-operative for mixing the feeds to specifications, Farm Fare (Transvaal) for providing slaughtering facilities and Mr L Dunn, techinical assistant.

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EXOPHTHALMOS IN A HORSE RESULTING FROM AN ADENOCARCINOMA OF THE FRONTAL SINUS

F W G HILL,* J E MOULTON+ and P H SCHIFF*

ABSTRACT

A fifteen-year-old thoroughbred gelding with exophthalmos of the left eye, was found at necropsy to have an adenocarcinoma of the left frontal sinus. The tumour extended caudally through the cribriform plate into the orbit, displacing the eyeball anteriorly.

Key words: Equine, adenocarcinoma, frontal sinus

Hill F.W.G.; Moulton J.E.; Schiff P.H. **Exophthalmos in a horse resulting from an adenocarcinoma of the frontal sinus.** *Journal of the South African Veterinary Association* (1989) 60 No. 2, 104-105 (En). Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 67, Harare, Zimbabwe.

INTRODUCTION

A variety of tumours have been reported in the nasal passages and paranasal sinuses of the horse[1,2,3,6,7] but such lesions remain relatively uncommon[4]. Tumours of the paranasal sinuses arise almost as frequently as those from the nasal cavity[4]. Squamous cell carcinomas predominate but adenocarcinomas are recorded from time to time and the maxillary sinus is a common site[4,7]. Presenting clinical signs, invariably referrable to the respiratory tract, are dyspnoea, facial swelling and chronic nasal discharge[6]. This report describes the clinical and pathological features of an adenocarcinoma originating in the left frontal sinus of a horse and producing exophthalmos of the left eye.

CASE REPORT

A fifteen-year-old Thoroughbred gelding was referred to the Veterinary Hospital of the University of Zimbabwe with a history of exophthalmos of the left eye having developed over a period of 2 months (Fig. 1), inspiratory and expiratory noises, a purulent and haemorrhagic discharge from the left nostril and a soft swelling over the left temporal fossa. There was no visible abnormality of the frontal or maxillary bones. Before admission, the animal had received courses of topical antibiotic eye ointment (Chlornicol, CAPS, Zimbabwe) and a course of 3 intravenous injections of 30ml isopyrin and phenylbutazone preparation (Tomanol, Byk Gulden S.A. (Pty) Ltd) without any noticeable improvement. The horse had not been ridden for several months and was kept stabled with other horses, all of which appeared clinically normal. There was no previous history of trauma to the head or of respiratory disease.

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Received: August 1988 Accepted: January 1989

On clinical examination, the horse was alert and in good physical condition. There was a purulent blood-tinged discharge at the left nostril and at rest, considerable inspiratory effort with flaring of the nostrils. The left eye had a serous ocular discharge and the eyelids just met when closed over the protruding globe. Intraocular tension, determined by digital pressure, was similar in both eyes. The sclera, conjunctiva and cornea were normal, but the pupillary light and consensual reflexes in the left eye were sluggish compared with the right. The optic disc and associated blood vessels appeared atrophied. There was decreased resonance on percussion of the left frontal sinus. When moving, the horse failed to see objects on the left side. Standing, lateral and dorsoventral radiographs of the skull revealed a diffuse dense shadow in and behind the left orbit and extending into the left frontal sinus. The nasal septum was intact and the left nasal cavity contained material of increased density. Endoscopic examination of the left nasal chamber revealed profuse purulent exudate and a small polyp situated posteriorly on the lateral wall. No abnormality was observed in the right nasal chamber. On the basis of the clinical and radiographic findings, a tentative diagnosis was made of retrobulbar neoplasia, with possible invasion of the frontal sinus. Euthanasia was performed on the horse.

At necropsy, lesions were found to be confined to the head. A large, red, homogeneous mass occupied almost the entire left frontal sinus and extended laterally into the right frontal sinus and caudally through the cribriform plate. The mass surrounded the optic nerve and the posterior orbit, displacing the left eyeball forwards. Tumour tissue also extended along the optic nerve and terminated in a large mass which compressed the cerebrum at the base and left side of the cranial cavity. The pituitary gland was normal on external examination, but on incision contained a large centrally located cystic cavity surrounded by a mass of hyperaemic and lobulated glandular-like tissue. The left frontal and maxillary sinuses were inflamed and contained a thick purulent exudate. The nasal septum was not affected and the regional lymph nodes appeared grossly normal.

Samples of lung, myocardium, liver, spleen, kidney, submandibular, parotid and retropharyngeal lymph nodes, pituitary, medulla oblongata, optic nerve and neoplastic tissue from various sites were fixed in 10% buffered formalin, routinely processed and embedded in wax blocks, and then sectioned and stained with haematoxylin eosin and the periodic acid-Schiff (PAS) reaction. Significant pathological changes were confined to the tumorous mass and associated tissues. The mass was composed of epithelial cells forming clusters, cords and acini. The tumour cells had central round nuclei and vacuoles in the cytoplasm which were PAS-negative. Mitotic figures were frequently encountered. The tumour cells were separated by delicate connective tissue fibres and blood vessels around which there was haemorrhage. Part of the tumour in the right frontal sinus showed an inflammatory reaction with numerous polymorphonuclear leucocytes. In places there were lymphocytes and plasma cells or large areas of necrosis and haemorrhage.

Within the sella turcica the neoplastic tissue consisted of large spindle-shaped cells with no cytoplasmic vacuoles, arranged in uniform clusters and separated by a thin stromal meshwork containing capillaries and neutrophils. There were focal areas of haemorrhage, haemosiderosis and cholesterol accumulation. Here the histological features suggested an adenoma of the pars intermedia. There was chronic suppurative inflammation of the pituitary tentorium induced by the surrounding tumour mass but the remaining glandular tissue was normal although hyperemic.

In the left eye there was severe suppurative inflammation of the sclera associated with haemorrhage and thrombosis. The inflammation extended into the extrinsic eye muscles. There was no evidence of metastases in the submandibular, parotid or retropharyngeal lymph nodes, but all contained numerous plasma cells.

The histological appearance of the paranasal tumour was that of a well defined adenocarcinoma of the frontal sinus and a coincidental finding was the pituitary adenoma.

DISCUSSION

Although there are only a few reports on neoplasia of the frontal sinus in the horse, most mention that a local distortion or softening of the bone are common clinical findings. This particular tumour was unusual, since the predominant clinical sign was ocular rather than nasal. The risk for nasal and paranasal neoplasms generally increases as

animals age and is greatest in horses 15 years and older[7].

Due to the lack of clinical involvement of the frontal bone, trephination was not carried out. The decreased resonance of the left frontal sinus was believed to be due mainly to the tumour mass and secondarily to inflammation, necrosis and exudation from the adjacent endothelium.

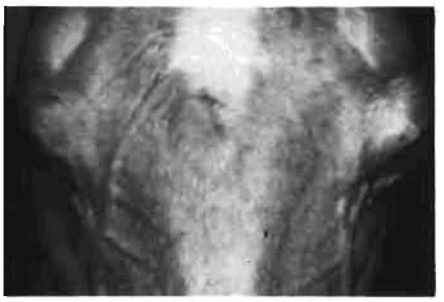

Fig. 1: **Exophthalmos of the left eye and bulging of the supraorbital fossa. There is no obvious distortion of the left frontal sinus**

Moulton[8] describes adenocarcinoma of the equine frontal sinus to consist of mucin cells forming highly differentiated acini and ductules, with a papillary or palisade pattern. Areas of squamous metaplasia and a resemblance to thyroid follicles may also be found. Local invasion and destruction is a common consequence of tumours within the frontal sinuses and metastases are rare[4]. Retrobulbar tumours are uncommon but have been described[5, 9] and include melanoma, astrocytoma, lipoma, medullo-epithelioma, retinoblastoma and neurofibroma. Whether such tumours can and do invade the adjacent sinuses has not been reported.

Pituitary adenoma of the *pars intermedia* is a relatively common tumour in the aged horse and may grow to a considerable size, compressing the dorsally situated hypothalamus. Affected horses may exhibit a variety of clinical signs. In the present case the pituitary tumour was not grossly enlarged and there were no attributable clinical signs.

ACKNOWLEDGEMENT

We are grateful to Professor D F Kelly for confirming the histopathological description of the tumours and reading the manuscript, and to Mr J V Dele-Hoffman for the photograph.

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THE ANGIOCARDIOGRAPHIC DIAGNOSIS OF A PERSISTENT TRUNCUS ARTERIOSUS IN A FOAL

P F STEYN*, PATRICIA HOLLAND** and J HOFFMAN***

ABSTRACT

Persistent truncus arteriosus is a relatively rare cardiac anomaly which is associated with a single large artery arising from the ventricles. An interventricular septal defect is invariably present. The vessel gives origin to the pulmonary trunk, aorta and coronary arteries. A description of the angiocardiographic diagnosis of this condition is given as well as a general review of the relative developmental anatomy.

Key words: Foal, persistent truncus arteriosus, angiocardiography, embryology, radiography, cardiac anomaly

Steyn P.F.; Holland P.; Hoffman J. **The angiocardiographic diagnosis of a persistent truncus arteriosus in a foal.** *Journal of the South African Veterinary Association* (1989) 60 No. 2, 106-108 (En.) Department of Radiology, Texas Veterinary Medical Center, Texas A&M University, College Station, Texas, 77843, United States of America

INTRODUCTION

In truncus arteriosus a single vessel arises from the ventricles above a ventricular septal defect and gives origin to systemic, coronary and pulmonary arteries[5]. The pulmonary arteries can originate at different levels: with a separate pulmonary trunk, with common origin of pulmonary arteries (but no pulmonary trunk) or with separate origin of the pulmonary arteries[3]. Both ventricles act as a common pumping chamber and eject blood at systemic pressure into the truncus where mixing of the venous and arterial blood occurs. Truncus arteriosus has been diagnosed in equines on post mortem examination[2,4]. Sojka[6] discussed a case report of a foal with a persistent truncus arteriosus.

This report describes the ante mortem angiocardiographic diagnosis in a foal with a persistent truncus arteriosus with a pulmonary trunk arising separately from it.

CASE HISTORY

A quarter horse filly was presented immediately after birth to the Texas Veterinary Medical Center with dyspnoea and extreme exercise intolerance. On physical examination, a severe grade 5/6 holosystolic murmur was noted on both sides of the thorax. Tachypnoea and cyanosis were evident after exercise. On auscultation the lung fields were clear, the rectal temperature was 38,5°C, the pulse was 140 - 160 min^{-1} and the respiration rate 60 - 80 min^{-1}.

Echocardiography demonstrated a high ventricular septal defect with dextropositioning of the aorta. Right ventricular hypertrophy was suggested on the short axis view. The pulmonary trunk could not be identified.

When the filly was 3 weeks old a cardiac catheterisation and an angiocardiogram were performed to aid in the diagnosis of the suspected cardiac anomaly. Anaesthesia was induced with 5% and maintained on 2,5 to 3,5% Isofluorane and oxygen. Survey lateral thoracic radiographs were taken to establish the radiographic exposure technique. Cardiac catheterisation with a size 7 cardiac catheter (Cordis GJ 7 French 80cm Cardiac Catheter Cordis Corporation, Miami, FL) was achieved via the right jugular vein. The tip of the catheter was guided into the right atrium per fluoroscopy (Picker P-600 Retro Fluoroscope, Bay Shore, NY) and was then passed through the ventricular septal defect into the left ventricle. Fifty-eight ml of contrast medium (Renografin-76, Squibb, Princeton, NJ) were injected through the catheter using an angiographic injector (Medrad Mark VI Angiographic Injector, Pittsburg, PA) at 24 ml sec^{-1} at 32 kg cm^{-2} with a delay time of 1 s. Rapid serial radiography was achieved by means of a Puck Rapid Film Changer (Elema-Schonander, Stockholm, Sweden). Opacification of the aorta was visible, as well as a blood vessel in the position where a patent ductus arteriosus would be expected (Fig 1). The catheter was then withdrawn and introduced into the right atrium where contrast material was injected with the rapid injector. Immediate opacification of the aorta and the patent ductus arteriosus-like vessel was observed; the same angiocardiographic image of the major vessels leaving the heart was visible whether the contrast agent was injected into the left ventricle or the right atrium (Fig.2). The ascending aorta was noted with the dextroangiocardiogram, and positive contrast agent was seen to shunt from the right atrium to the left atrium through an atrial septal defect. At no time was a pulmonary trunk seen to exit the right ventricle. Blood gasses and pressures were obtained (Table 1). The pressures in the right and left ventricles were essentially the same. The pO_2 concentration was highest in the left ventricle and higher in the aorta than in the right ventricle.

Angiocardiographic findings included a high ventricular septal defect, a functional patent foramen ovale and a common truncus arteriosus originating from the right ventricle and giving origin to the pulmonary trunk, aorta and the coronary arteries. Both ventricular free walls were of essentially the same thickness. At this stage, the diagnosis of a persistent truncus arteriosus was made. The foal was euthanased and a necropsy was performed. The right ventricle was moderately dilated and the right ventricular free wall was hypertrophied, measuring 19mm in thickness as compared to 16mm for the left ventricular free wall. A 14 x 31mm interventricular septal defect was located high in the muscular septum just adjacent to the atria and resulted in communication between the left and right ventricles. A single major arterial trunk left the heart, arising predominantly from the right ventricle, but slightly overriding the interventricular septum. This vessel had a functional valve with 3 well-developed leaflets. Coronary arteries branched from the truncus arteriosus near its origin in the vicinity of the valve. The pulmonary trunk branched from the truncus arteriosus approximately 70 mm from the base of the heart and gave rise to the left and right pulmonary arteries (Fig. 3). The brachiocephalic trunk branched from the truncus arteriosus at about the same level as and opposite to the pulmonary trunk. A patent foramen ovale was also noted (Fig 4).

DISCUSSIONS

Embryologically the aorta and the pulmonary artery originate from the same structures: the bulbus and the truncus. Two prominent longitudinal thickenings (the bulbar ridges) arise from the endocardial lining during the early developmental stages in the heart. The bulbar ridges then meet and fuse, creating a septum that divides the truncus and the unabsorbed portion of the bulbus into an aorta and a pulmonary trunk. The ventricular septum develops from the base of the common ventricle and increases in height, thereby creating 2 ventricles. For a short time the septum is incomplete proximally where the interventricular foramen is situated. The proximal portion of the interventricular

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Received: August 1988 Accepted: December 1988

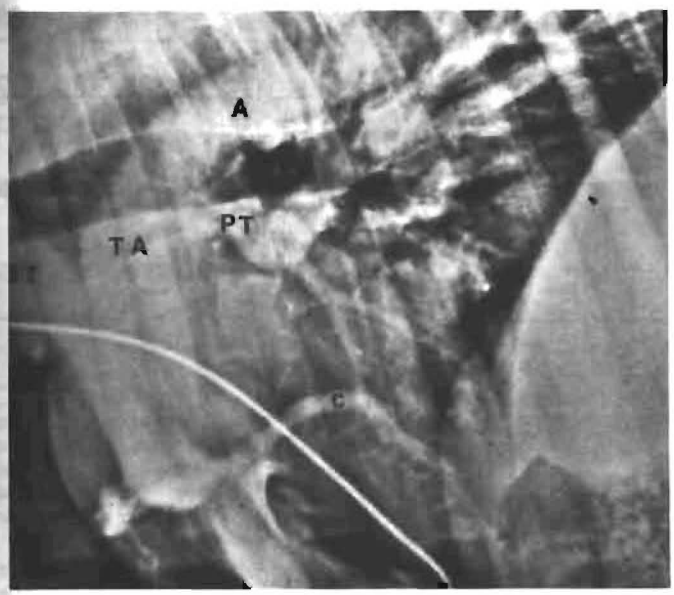


Fig. 1: Laevo-angiocardiogram: the catheter tip is situated in the left ventricle. Note the opacification of the truncus arteriosus (TA), the coronary arteries (c), the pulmonary trunk (PT), the aorta (A), and the brachiocephalic trunk (BT)

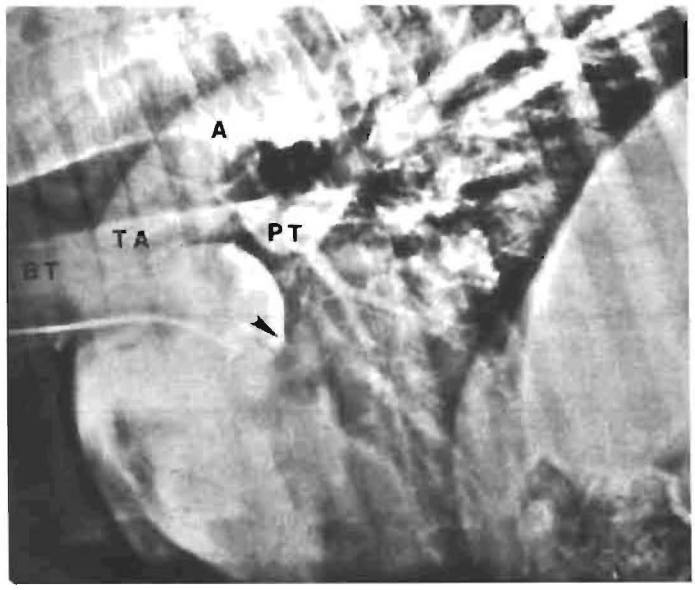
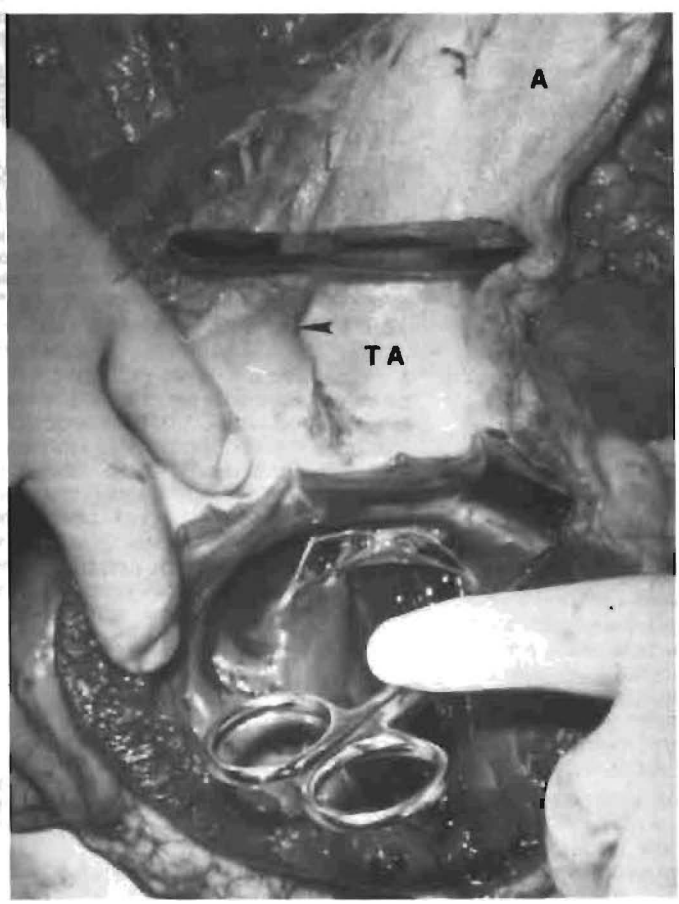


Fig. 2 Dextro-angiocardiogram: the catheter tip is in the right atrium. Note the opacification of the right atrium, right ventricle, truncus arteriosus (TA), pulmonary trunk (PT), brachiocephalic trunk (BT), aorta (A) and the patent foramen ovale (arrow head). Compare this with Fig. 1 and note the resemblance. At no stage is a pulmonary trunk seen to originate from the right ventricle

Fig. 3: Necropsy specimen; the right ventricle has been opened. The forceps are situated in the pulmonary trunk orginating from the truncus arteriosus (TA) and the scissors indicate the high ventricular septal defect. The origin of the brachiocephalic trunk (arrow head) and the aorta (A) are marked

Fig 4 Necropsy specimen: the left ventricle (LV) has been opened. The scissors are located in the high ventricular septal defect and the probe (arrow) indicates the patent foramen ovale opening into the left atrium (LA). Note the absence of an ascending aorta originating from the left ventricle



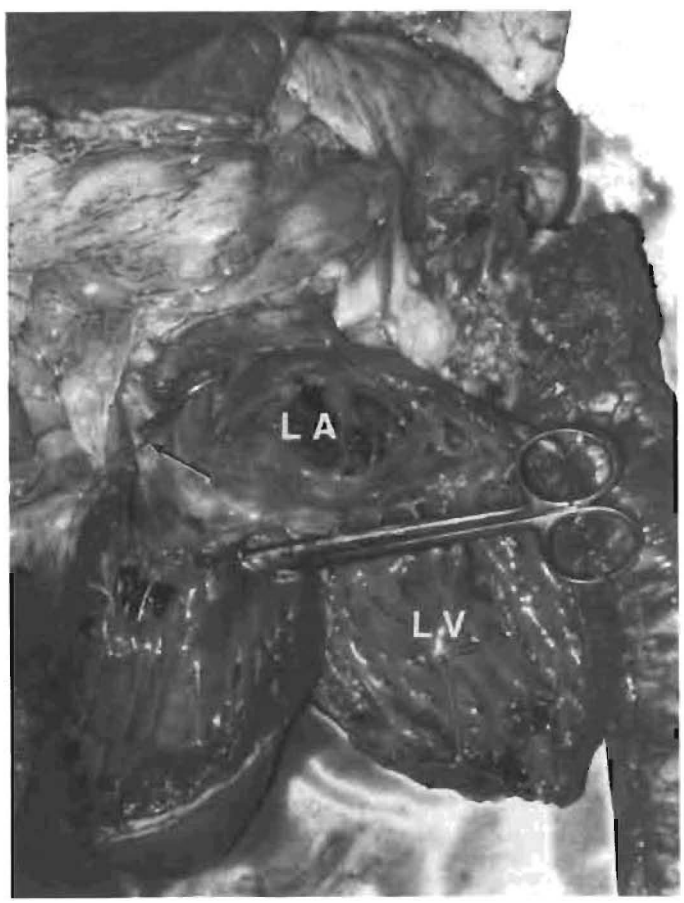


Table 1: **Partial gas pressures, pH of the blood and systolic/diastolic pressures in the right ventricle, right atrium, aorta and left ventricle in a foal with persistent truncus arteriosus**

	Right atrium	Right ventricle	Aorta	Left ventricle
pO_2 mm Hg	39,8	39,9	52,9	60,8
pCO_2 mm Hg	56,8	57,1	53,1	58,5
HCO_3 mmol ℓ^{-1}	27,0	25,7	26,1	27,4
pH	7,285	7,262	7,301	7,280
Pressure mm Hg systolic/diastolic	15/10	90/9	70/5	91/10

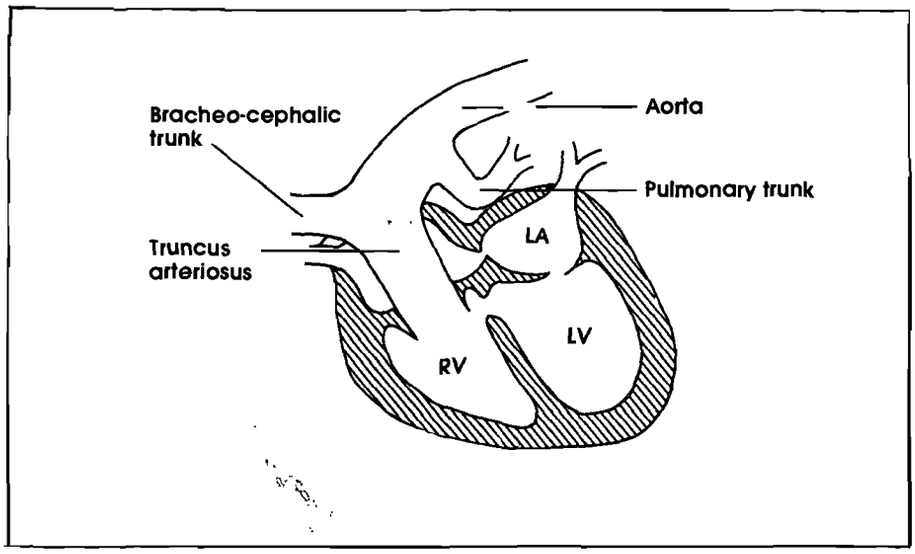


Fig. 5: **Schematic drawing of the heart of a three-week-old foal with a persistent truncus arteriosus arising predominantly from the right ventricle (RV.) The high ventricular septal defect and atrial septal defect (patent foramen ovale) are not annotated. Left atrium (LA) and left ventricle (LV).**

foramen later fuses with the proximal bulbar septum. The proximal part of the bulbus (which forms part of the pulmonary trunk) is incorporated into the right ventricle[1].

In this foal, apparent incomplete division of the proximal portion of the truncus and the bulbus resulted in a persistent truncus arteriosus overriding the ventricles, but situated predominantly over the right ventricle (Fig 5). Because the bulbus was involved in this anomaly, the interventricular septum did not close; this resulted in the high ventricular septal defect.

The pO_2 of the aorta was higher than the pO_2 of the right ventricle because the oxygenated blood from the left ventricle followed the path of least resistance, i.e. up the truncus arteriosus and not into the right ventricle per se because the pressures of these two chambers were equal. The high pressure in the right side of the heart was presumably responsible for the nonclosure of the foramen ovale in the atrial septum.

The ante mortem diagnosis of a persistent truncus arteriosus is most reliably made by angiocardiography. Echocardiography is often not rewarding in this condition[7] and therefore it is important that angiocardographic findings be well described and documented.

ACKNOWLEDGEMENT

The authors wish to thank Ms. Joni Elslander for her technical assistance.

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SUSPECTED VETCH *(VICIA BENGHALENSIS L)* POISONING IN A FRIESLAND COW IN THE REPUBLIC OF SOUTH AFRICA

JENNIFER R. GREEN* and JOAN E. KLEYNHANS**

ABSTRACT

The clinical findings, treatment and pathological changes are described in a case of suspected vetch *(Vicia benghalensis L.)* poisoning in a Friesland cow in the Clanwilliam district, Republic of South Africa. These were characterised by a severe pruritic dermatitis, granulomatous myocarditis and a nephritis.

Key words: **Cattle, vetch poisoning *(Vicia benghalensis L.)*, dermatitis**

Green, Jennifer R.; Kleynhans, Joan E. **Suspected vetch (Vicia benghalensis L.) poisoning in a Friesland cow in the Republic of South Africa** *Journal of the South African Veterinary Association*, (1989) 60 No. 2 109-110 (Eng.) Regional Veterinary Laboratory, Private Bag X 5020, 7600 Stellenbosch, Republic of South Africa

INTRODUCTION

Vetch is a legume, planted as artificial pasture for hay or winter grazing. Various forms of vetch poisoning have been described. The seeds and plant may contain cyanogenetic glycocydes[3 4] as well as substances which induce toxic hepatitis or haemolytic anaemia[3]. Photosensitisation has accompanied the ingestion of common vetch *(Vicia saliva L.)*[3]. In humans, ingestion of the fava bean *(Vicia faba L.)* can cause an acute haemolytic crisis[3].

Three syndromes have been reported in cattle grazing hairy vetch[2]: a)nervous signs and rapid death; b)subcutaneous swellings, respiratory signs and death after 12 to 15d; c)the third syndrome occurs only after the grazing of vetch for 2 to 6 weeks and is characterised by a severe pruritic dermatitis, conjunctivitis, dyspnoea, diarrhoea, occasional abortions and a high mortality rate.

Two outbreaks of suspected *Vicia* toxicity in the Humansdorp district, eastern Cape Province, were reported by Burroughs et al[1]. These were very similar to the third syndrome described by Panciera et al[2 3].

Vicia toxicity was suspected in 6 Friesland cows in the Riebeeck Wes area in October 1983 (D.J. Schneider, Regional Veterinary Laboratory, Stellenbosch, unpublished data). These cows became thin and listless, their milk production decreased and they all had erosive pruritic skin lesions. The animals had been grazing a pasture containing predominantly *Vicia hisutu* for one month.

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** Private Practitioner, Clanwilliam

Received: October 1988 Accepted: February 1989

CASE REPORT

During July 1987 the private practitioner was summoned to a 17-cow Friesland dairy herd on the Olifants River, about 8 km north of Clanwilliam. The irrigated pastures that the cattle grazed, had been divided into 5 one-hectare camps. One contained lucerne and the others vetch *(Vicia benghalensis L.)*. The latter had been planted at the beginning of May. The cattle had grazed the 5 camps, rotating from one to the other from the middle of June. By July the vetch was well established and was the predominant plant in the 4 camps.

One mature cow had a severe pruritis and dermatitis manifested as small raised nodules of 2 to 4 mm, spread evenly over her entire body. Black and white skin was affected to the same degree and the nodules were about 20 to 30 mm apart. The pruritis was so severe that erosive skin lesions had developed on the cow's forelegs as a result of her constant licking and biting. An allergy with unknown aetiology was diagnosed and the cow was treated with an antihistamine preparation which brought minimal relief.

Three days later the cow was seen again. The skin had been rubbed raw in places and the appetite and milk production of the animal were depressed. She was treated with a long-acting penicillin and a corticosteroid preparation. Thereafter the skin condition and appetite of the animal improved gradually.

The cow was not seen again until the private practitioner was called out on Day 21. By this time the animal had lost condition, was lying down with the head on the flank, was very weak and had a subnormal temperature. After intravenous fluids, vitamins, corticosteroids and antibiotic treatment the cow initially showed some improvement but died later the same day.

The remaining 16 cows were healthy and their milk production was normal.

At necropsy, the most noticeable lesions were those in the kidneys. Whitish foci, about 2 to 3 mm in diameter, were spread evenly throughout the kidney. Apart from splenomegaly, the other organs were normal. The condition of the cow was poor and the skin lesions had started healing.

Specimens of the kidney, liver, heart spleen, lung and skin were collected and fixed in 10% buffered formalin. Sections were processed in a routine manner for histopathological examination and were stained with haematoxylin and eosin (HE).

In the kidney numerous foci of varying size, consisting of mononuclear cells as well as a few giant cell and eosinophils, were present in the interstitium (Fig. 1). In some tubules the epithelium was necrotic and there was hydropic degeneration of the remaining epithelial cells. A few tubules were mineralised and others had undergone cystic dilatation. Fibrosis of the interstitium was evident in some of these areas.

The heart lesions were characterised by marked infiltration of mainly mononuclear cells and eosinophils, but also of numerous giant cells. Focal fibrosis was present and a few myocardial fibres were mineralised.

Slight portal fibrosis, accompanied by mild bile ductular proliferation and mononuclear cell infiltration, was observed in the liver. Other changes included necrosis of individual or small groups of hepatocytes and hydropic degeneration of the parenchyma cells.

The spleen was moderately congested, haemosiderosis was evident in the red pulp, and the lymphoid nodules appeared to be enlarged.

The lesions in the lungs consisted of patches of acute pneumonia. The alveoli in these areas contained serous exudate and the alveolar walls were thickened by eodema and infiltration of eosinophils.

Hyperkeratosis, hyalinisation of the collagen in the dermis, a mild vasculitis and perivascular infiltration of eosinophils were evident in the skin.

DISCUSSION

Similar to the cases reported in the Humansdorp district[1], this cow developed clinical signs after grazing lush vetch for about 1 month. The clinical signs and pathological changes were similar to those described by Burroughs et al[1], but the liver was not as severely affected and no vascular changes were found in the kidneys and myocardium.

ACKNOWLEDGEMENTS

We would like to thank Dr. J.A. Neser, Miss. A.M.D. Kriel, Mrs. R. de Kok and Mr. P van der Merwe for their assistance.

We are grateful to the Directorate of Veterinary Services, Pretoria, for permission to publish this article.

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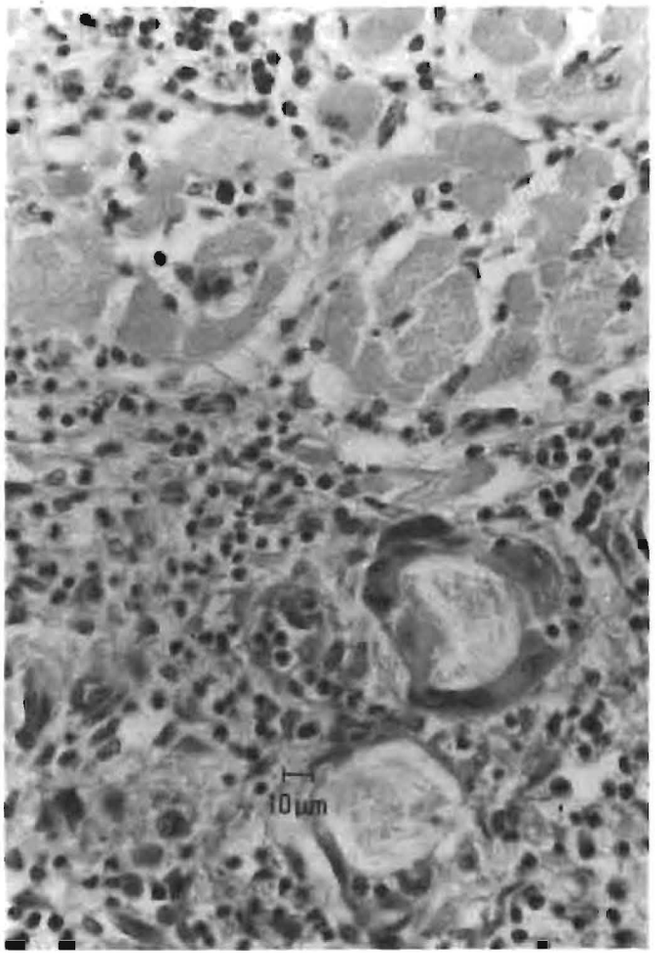

Fig. 1 **Myocarditis, characterised by mononuclear cell, eosinophil and giant cell infiltration. HE X 300**

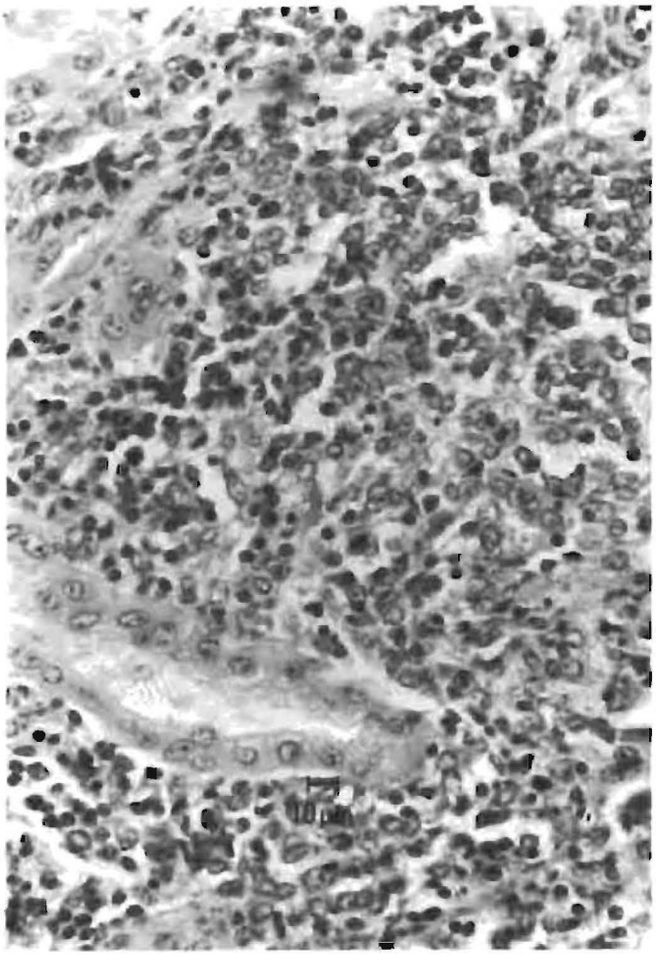

Fig. 2 **Focal interstitial lymphocyte, monocyte and eosinophil infiltration in the kidney. HE X 300**

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CONGENITAL MALFORMATION AND VARIATION OF THE LUMBAR VERTEBRAE IN A DOG

R M KIRBERGER*

ABSTRACT

The clinical, radiological and anatomical changes in a 6½-month-old Fox Terrier bitch with congenital malformation and variation of the lumbar vertebrae are described. The dog had 5 lumbar vertebrae and the first lumbar vertebra was malformed resulting in a dorsal hemivertebra, kyphosis and spinal cord compression. The possible pathogenesis of the dorsal hemivertebra is mentioned.

Key words: Congenital, vertebra, malformation, variation, hemivertebra, dog

Kirberger R.M. Congenital malformation and variation of the lumbar vertebrae in a dog. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 111-112 (En.) Radiology Section, Department of Surgery, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.**

INTRODUCTION

Congenital anomalies frequently occur in the vertebral column of the dog[3] Vertebral variations are differences in the number of vertebrae. Malformations are deformities or anomalies of the vertebral bodies and/or vertebral arches and their processes Malformations and variations often occur simultaneously and are commonly seen at transitional areas of the vertebral column

CASE HISTORY

A 4-month-old intact Fox Terrier bitch was initially presented with a complaint of mild paresis and a painful mild swelling over the thoracolumbar area. On clinical examination, the dog was found to be alert and in good condition A neurological examination revealed decreased proprioception of the back legs with the right leg being slightly more affected, accompanied by occasional bunny-hopping The thoracolumbar region was painful on pressure application Radiographs were taken and the patient was discharged with a diagnosis of myelopathy resulting from a dorsal hemivertebra of the first lumbar vertebra

Ten weeks later, the patient was readmitted with a history of worsening clinical signs. Neurologically the patient then showed increased loss of proprioception of the back legs as well as deficits in hopping and placing postural reactions Lateral and ventrodorsal radiographs were taken of the whole vertebral column. The patient was euthanased at the owner's request.

RADIOLOGICAL FINDINGS

The following radiological abnormalities were detected (Fig 1). Only 5 lumbar vertebrae were present; the first lumbar vertebra was lying dorsally to the thirteenth thoracic and second lumbar vertebrae, the thirteenth thoracic and second lumbar vertebrae were in contact with each other and wedged dorsally, resulting in kyphosis, the spinous - and transverse processes as well as 80% of the vertebral body of the first lumbar vertebra were absent; and, the cranial opening of the *canalis vertebralis* of the first lumbar vertebra had a diameter of 3 mm compared to the caudal opening of 8 mm The rest of the vertebral column was radiologically normal

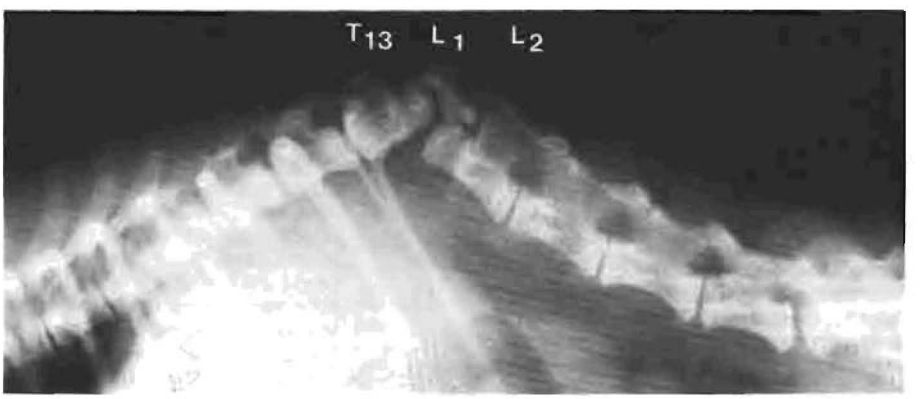

Fig. 1 **Lateral radiograph of the thoracolumbar spine**

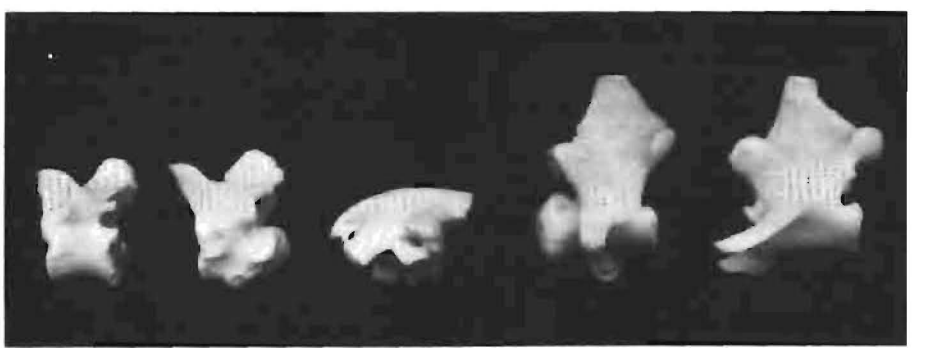

Fig. 2· **Lateral view of T_{12}, T_{13}, L_1, L_2 and L_3**

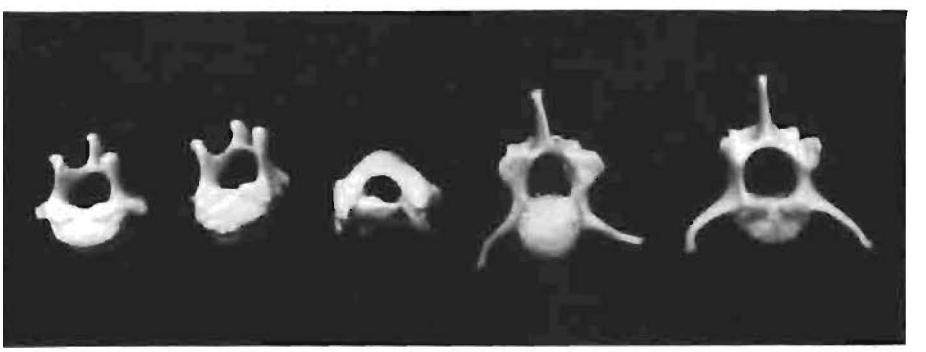

Fig. 3· **Cranial view of T_{12}, T_{13}, L_1, L_2 and L_3**

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Received, November 1988 Accepted February 1989

MACROSCOPIC FINDINGS

The affected section of the vertebral column was prepared using the enzyme-active detergent technique as described by Mooney et al.[2].

The thirteenth thoracic vertebra showed mild malformation of the pedicles and lamina. The caudal extremity of the vertebral body was angled at 45° in order to articulate with the first and second lumbar vertebrae (Fig 2 and 3). The abnormalities found in the first lumbar vertebra correlated well with the radiological findings. In addition, the cranial vertebral opening was narrower on the right side than the left side; the cranial and caudal articular processes were about 50% of their normal size and faced cranioventrally and caudally respectively instead of craniomedially and caudolaterally; the caudal vertebral incisures were closed to form foramina.

The cranial extremity of the vertebral body of the second lumbar vertebra showed 2 distinct articular facets. One quarter of its surface faced craniodorsally to articulate with the first lumbar vertebra and 3/4 of its surface faced cranially to articulate with the thirteenth thoracic vertebra.

DISCUSSION

This case showed a decrease in the number of lumbar vertebrae from 7 to 5 resulting in 25 presacral vertebrae. This variation is a rare occurrence[3].

Vertebral malformations of ectodermal origin result in neural arch defects like spina bifida, whereas those of mesodermal origin commonly result in block vertebra or hemivertebra. Hemivertebra is commonly seen in dogs, especially in the screw-tailed breeds, but the majority of cases are asymptomatic. According to Done[1] these commonly occur between the sixth and tenth thoracic vertebrae and he described 17 cases in which the hemivertebra had resulted in clinical signs.

Classification of malformations is difficult since each vertebra is composed of several individual parts and developmental errors may cause disturbances of other parts. Schmorl & Junghanns[4] classified vertebral malformations in humans and the following classification for dogs, based on their classification, is suggested by the author:

A. Abnormal shape of the vertebral body

These are common in the dog and are represented by:

1. Cleft vertebra in the sagittal plane resulting in paired hemivertebra (also known as "butterfly" vertebra).
2. Lateral hemivertebra resulting from hemimetameric segmental displacement of somites during transformation of the primitive segments into final segments. This transformation often results in scoliosis.
3. Dorsal hemivertebra due to absence of ossification of the ventral half of the vertebral body. This results in the typical cuneiform shape of the vertebral body as seen on lateral radiographs.

B. Absence of a vertebral body resulting in a dorsal hemivertebra

This is rare in animals and results from:

1. Agenesis of the vertebral body's prechondral anlage. This is extremely rare in humans and is often accompanied by agenesis of vertebral segments, together with malformation of other organ systems.
2. Absence of vertebral body ossification. This may be total, resulting in the ossification centres of the pedicles fusing ventrally and in kyphosis after birth as the cartilagenous tissue is unable to bear the dynamic stresses placed on it. This abnormality is probably due to inadequate vascularisation of the cartilage.

Whether agenesis of the vertebral body's prechondral anlage or total absence of the vertebral body ossification centre was the cause of the dorsal hemivertebra in this case, is impossible to say as the end result in both cases is much the same.

CONCLUSION

Congenital malformations of the vertebral column rarely cause clinical signs of disease except in cases of dorsal hemivertebra where deviation of the vertebral segment may result in myelopathy from vertebral canal stenosis. This is believed to be the first described case of dorsal hemivertebra accompanied by agenesis of 2 vertebrae in the lumbar region.

Radiological evaluation and a thorough knowledge of the possible aetiology of the deformity will assist the clinician in evaluating the case for a prognosis and possible surgical intervention.

ACKNOWLEDGEMENTS

The author wishes to thank Prof. C J Roos, Dr W L Berry, Mrs. C M S Liebenberg Mrs. H Smit for their assistance.

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AN EXPANSILE SECONDARY HYPOPHYSEAL MASTOCYTOMA IN A DOG

G N ECKERSLEY*, STELLA BASTIANELLO**, J VAN HEERDEN* and JUNE H WILLIAMS*

ABSTRACT

A 5-year-old mixed breed dog was presented with a history of depression and anorexia. Physical examination revealed a pharyngeal tumour and a neurological examination indicated the presence of a possible space-occupying lesion in the brain. Investigative procedures included a bloodsmear, impression smears and cytology of the pharyngeal tumour, haematology, chemical pathology, faecal analysis, urinalysis, electrocardiography, cerebrospinal fluid analysis, hormone assays and a computerised axial tomography scan. Results of these investigations revealed a round cell tumour in the pharynx, hypergammaglobulinaemia (34 g ℓ^{-1}), azotaemia (urea 8,6 mmol ℓ^{-1} and creatinine 170 μmol ℓ^{-1}), hypoalbuminaemia (20 g ℓ^{-1}), proteinuria, sinus bradycardia (heart rate 60 beats per min), increased concentration of protein in the CSF (1,1 g ℓ^{-1}), hypoadrenocorticism (base line cortisol <55 nmol ℓ^{-1}) and hypothyroidism (T4 <13 nmol ℓ^{-1}). The computerised axial tomography scan revealed a brain tumour in the region of the hypophysis. The dog was euthanased and a post mortem examination confirmed the presence of a pharyngeal tumour with apparent direct extension of the tumour into the brain. Both tumours were confirmed histologically as mastocytomas.

Key words: Dog, computerised axial tomography scan, secondary hypophyseal tumour, mastocytoma

Eckersley G.N.; Bastianello S.; Van Heerden J.; Williams J.H. An expansile secondary hypophyseal mastocytoma in a dog. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 113-116 (En.) Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa.**

INTRODUCTION

A review of the literature on mastocytomas[12-14] and brain tumours[1 2 4-11 15 17] in dogs, revealed no reference to the occurrence of mastocytomas in the brain. Mastocytomas represent ca. 13% of all skin tumours in dogs[13 14], and 6% of all tumours in dogs (Lombard according to Stannard & Pulley[14]). Although mastocytomas vary in biological activity, they should be considered potentially malignant due to their invasive nature[14]. Metastasis rarely occurs via the lymphatics and blood stream to regional lymphnodes, spleen, liver, kidneys and lungs[14]. The pharynx and larynx are extremely rare sites for primary or metastatic mast cell tumours in dogs[12].

A number of neoplasms affect the brain by extension from adjacent cranial sites or by growing through foramina or through bone itself to impinge on nervous tissue[14]. Tumours arising from the bones or cartilage of the skull may cause pressure on the brain. Other tumours which have been reported to affect the brain by means of direct extension are lymphosarcomas, malignant melanomas, fibrosarcomas, haemangiosarcomas, plasmacytomas, carcinomas of the nasal mucosa or conjunctiva and transmissable venereal tumour (TVT) of the eye[11 14].

Metastasis of neoplasms to the brain from extraneural sites via the blood have also been described. Examples include pulmonary carcinomas, adenocarcinomas, haemangiosarcomas, malignant melanomas, lymphosarcomas, fibrosarcomas, TVT and fibrosarcoma[1 11 14].

This is the first reported case of a secondary mastocytoma in the hypophysis of a dog.

CASE REPORT

A 5-year-old male Wire-haired Terrier cross, body mass 7 kg, was presented with a history of depression and anorexia. The abnormal findings on physical examination included the presence of a pharyngeal mass, depression, subnormal body temperature (37°C), bradycardia (heart rate 60 beats per minute), weakness, mild dehydration (estimated to be 5%), a grade II/VI systolic murmur over the mitral valve area and atrophy of both testicles. The dog exhibited dysphagia when force fed. A complete neurological examination revealed severe depression, head pressing, circling and generalised body tremors. The posture was fairly normal but the gait was ataxic and hypometric with circling and falling to the left and right. Hypoalgesia was also present. Cranial nerve examination revealed bilateral absence of the menace reflexes, pupillary light reflexes and consensual reflexes. Ptosis and ventro-lateral strabismus were present in both eyes, but were more severe in the right eye. Evaluation of the neck, forelimb and hindlimb reflexes were normal but mild attitudinal and postural deficits were evident. The neurological examination indicated a lesion in the region of the optic chiasma, hypophysis, hypothalamus and thalamus. The limbic system and oculomotor nerve function were deranged.

The results of further investigations are listed in Table 1. Analysis of these results (Table 1) revealed various problems which are summarised in Table 2.

The CAT scan, (Somatom 2) (Fig 1) confirmed the presence of a brain tumour in the region of the pituitary gland involving the optic chiasma and causing pressure on the hypothalamus and brain stem. This resulted in distortion of the brain tissue and lateral ventricles. Contrast studies with Iopamidol (Jopomiron, Berlimed) enhanced the tumour outline.

Because of the apparent direct extension or metastasis of the pharyngeal tumour to the brain and the poor prognosis of patients with extensive brain tumours, the dog was euthanased.

A complete post mortem examination was performed. Impression smears were taken from the cut surface of the pharyngeal mass and stained with Giemsa for light microscopic examination. Selected sections of various tissues as well as of the pharyngeal and hypophyseal neoplasms were taken in 10% formalin for histopathological examination. The sections were routinely processed, cut and stained with haematoxylin and eosin (H&E). Selected sections of the neoplastic masses were stained with toluidine blue, Giemsa or the PAS method, to examine for the presence of cytoplasmic mast cell granules.

Macroscopic post mortem findings

A large (3x2x1cm), well circumscribed mass, creamy white in colour and of soft consistency was firmly attached to the roof of the nasopharynx. A large (2x1,5x1cm), well circumscribed, light brown mass was present in the hypophyseal region, extending from the rostral part of the optic chiasma to the level of the transverse fibres of the pons. This extension into the diencephalon resulted in pressure atrophy or necrosis of the hypothalamus and thalamus. The presence of the tumour also resulted in distortion of the thalamus with partial occlusion of the lateral ventricles (Fig 2).

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Received: November 1988 Accepted: February 1989

Table 1 **Pertinent laboratory findings in the dog with a mastocytoma in the hypophysis***

Laboratory test	Salient features
Haematology	White cell count 9,8 x 10^9 ℓ^{-1} (6-15) Neutrophils (mature) ,30 (,60 - ,80) Neutrophils (immature) ,05 Lymphocytes ,42 (0,12-0,3) Monocytes ,02 (,03 - ,10) Eosinophils ,21 (,02 - ,10) Platelets 126 x 10^9 ℓ^{-1} (200 - 500)
Chemical pathology:	Urea 8,6 mmol ℓ^{-1} (3,6 - 8,9) Creatinine 170 μmol ℓ^{-1} (133) Total protein 79 g ℓ^{-1} (53-75) Albumin 20 g ℓ^{-1} (25-35) Glucose 3,8 mmol ℓ^{-1} (3,3 - 5,5)
Protein electrophoresis	Gamma globulins 34,1 g ℓ^{-1} (6)
Radioimmunoassays	Cortisol < 55 nmol ℓ^{-1} (> 100) T4 < 13 nmol ℓ^{-1} (> 24)
Urinalysis	Proteinuria
Electrocardiogram	Sinus bradycardia (heart rate 60 beats min^{-1})
Cerebrospinal fluid analysis	Protein 1,1 g ℓ^{-1} (0,2-0,4)
Computerised axial tomography scan	A large tumour in the region of the pituitary gland was clearly visible.

* Normal parameters in brackets

Table 2: **Masterproblem list***

1. Neutropaenia
2. Lymphocytosis
3. Monocytopaenia
4. Eosinophilia
5. Thrombocytopaenia
6. Azotaemia
7. Hyperproteinaemia
8. Hypoalbuminaemia
9. Hypoglycaemia
10. Hypergammaglobulinaemia
11. Hypoadrenocorticism
12. Hypothyroidism
13. Proteinuria
14. Sinus bradycardia
15. High level of protein in the CSF

* Only abnormal laboratory parameters are listed here

Histopathological findings

Examination of the impression smear of the pharyngeal mass revealed masses of round cells of varying size which were mostly clumped together. The cells had predominantly round, central to eccentric, moderately vesicular nuclei and basophilic cytoplasm. No distinct granules could be seen in any of the neoplastic mast cells (Fig. 3). Eosinophils were scattered throughout the smear whereas neutrophils occurred only rarely.

The pharyngeal tumour was not encapsulated and consisted of neoplastic cells which extended to the submucosa, leaving a rim of unaffected connective tissue between the stratified squamous epithelium and the neoplastic mass (Fig. 4). The tumour had grown expansively, compressing the surrounding areolar connective tissue and salivary glands but

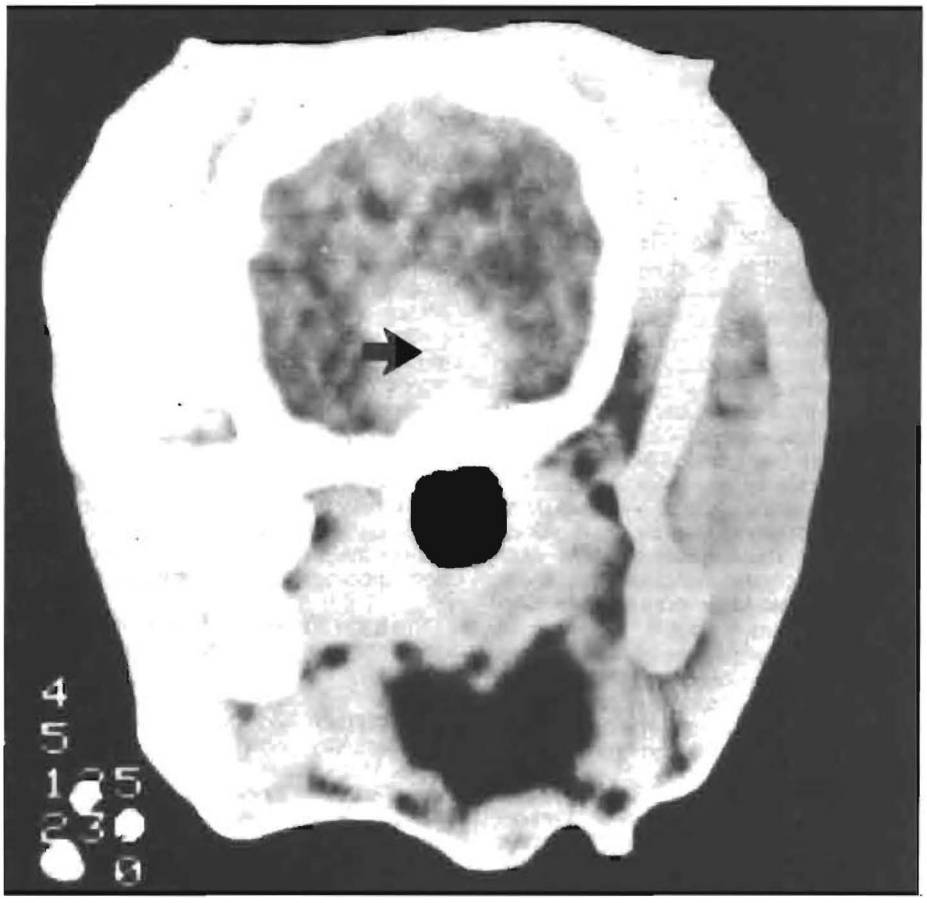

Figure 1: **Contrast enhanced transverse CAT scan of the brain of a 5-year-old male Wire-haired Terrier cross revealing a mass (arrow) in the hypophysis and hypothalamus area**

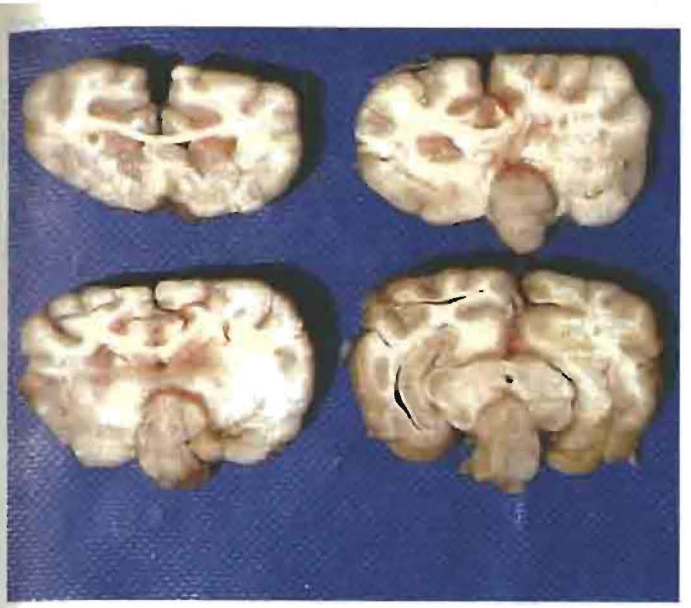

Figure 2: **Transverse sections of the formalin-fixed brain. The tumour is clearly visible on the ventral side of the brain in the region of the hypophysis and hypothalamus**

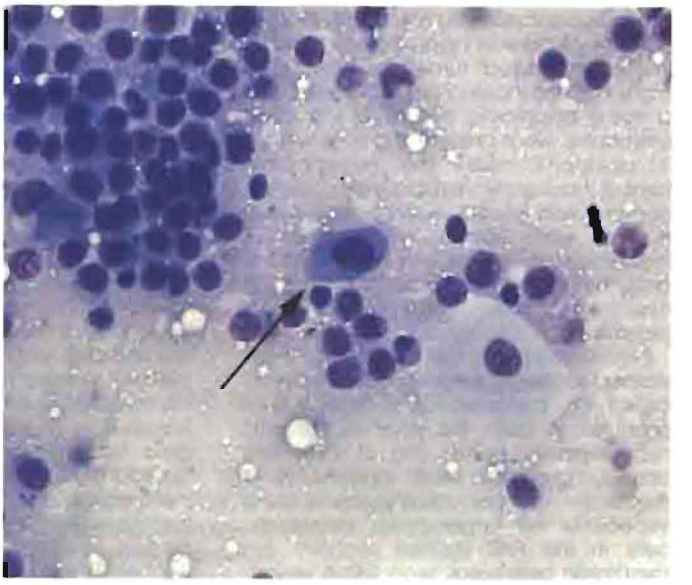

Figure 3: **Impression smear of the pharyngeal tumour. Note the typical mast cell (arrow) and epithelial cell. 5% Giemsa x 400**

Figure 4: **Pharyngeal tumour. Note band of unaffected connective tissue below the epithelium H & E x 100**

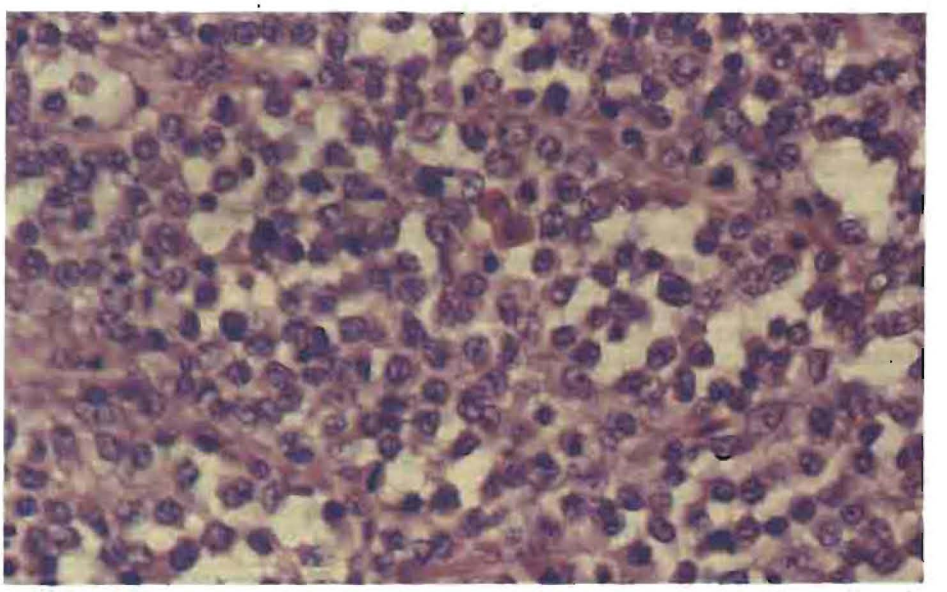

Figure 5: **Hypophyseal tumour. Note the tumour cells and 2 remaining acidophil cells H & E x 400**

incorporating portions of the salivary glands. Remnants of nerve fibres could also be seen within the tumour which was moderately vascularised and composed of sheets of cells with fine to coarse connective tissue fibres. At higher magnification the neoplastic cells were mostly round in shape. The cytoplasm varied from eosinophilic to basophilic and was scant to moderate in amount. The cells varied in size. The nuclei were moderately to highly vesicular and varied in size and shape from round to oval. Some of the nuclei were indented. The mitotic index was low (averaging 2 per 400 x magnification field.) No distinct granules were visible in the H&E stained sections, but the cytoplasm had a finely speckled appearance. Eosinophils were present scattered throughout the tumour singly or in groups of 5 to 30.

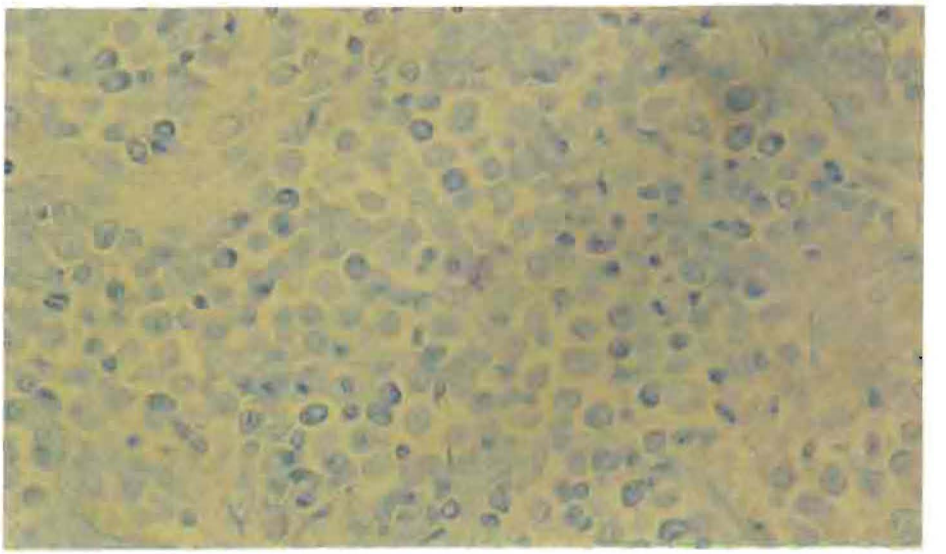

Figure 6: **Hypophyseal tumour. Note the metachromatic granules. Toluidine blue x 400**

The hypophyseal tumour consisted of cells very similar in appearance to those in the pharyngeal tumour, except for a higher mitotic index (5 per 400 x field) and a more anaplastic nature of the cells. Eosinophils were present (Fig 5) as well as 2 acidophil cells. The hypophyseal tumour had grown expansively, causing compression of the surrounding hypothalmic white matter. At the rim of the tumour, the tissue was fairly vascular and there was perivascular cuffing by neoplastic cells, lymphocytes and plasma cells.

Examination of the toluidine blue-stained sections revealed a few isolated cells with metachromatic cytoplasmic granules in both the pharyngeal and hypophyseal tumours (Fig 6). Fine PAS positive granules were present in a moderate number of neoplastic cells especially the larger more anaplastic cells in the PAS stained sections. No neoplastic cells were seen within vessels at either site.

The kidneys showed mild mesangioproliferative glomerulonephritis with mild interstitial plasmacytic/lymphocytic nephritis and mild interstitial medullary fibrosis.

DISCUSSION

The neoplasms in the pharynx and hypophysis were diagnosed as anaplastic, poorly differentiated malignant mastocytomas. The diagnostic features were: presence of a rim of normal non-neoplastic tissue below the pharyngeal mucosa, basophilia of the neoplastic cells, predominantly round shape of the cells, nuclei being mostly central in position, presence of eosinophils, and presence of metachromatic (through few) basophilic or PAS positive granules in the toluidine blue, Giemsa and PAS-stained sections. The above features are typical of mastocytomas as described by Scott et al.[13] and Stannard & Pulley[14]. Although mastocytomas metastasise via the lymphatics or the vascular system, neoplastic cells were not seen within the vessels in either the pharyngeal or hypophyseal sites. It is therefore speculated that metastasis to or direct extension to the hypophysis, may have occurred via the embryological remnants of Rathkes pouch[3]. This could further be supported by the expansive growth pattern of the neoplasms as opposed to the more usual invasive nature of mastocytomas[14]. The higher mitotic index and more anaplastic nature of the neoplastic cells in the hypophyseal tumour suggest that the neoplasm was more malignant at this site. In our opinion the primary site was in the pharynx.

Most of the abnormal clinical and neurological findings, as well as the abnormal laboratory parameters can be explained as resulting from the presence of the hypophyseal tumour. Destruction of the pituitary gland had resulted in secondary hypoadrenocorticism and hypothyroidism and associated abnormal haematological and blood chemistry parameters. Elevated concentrations of protein in the cerebrospinal fluid frequently occurs in association with brain tumours[5 8 11]. Sinus bradycardia is a recognised clinical entity in patients with space-occupying lesions in the brain[16]. Mast cell tumours are often associated with glomerulonephritis (with resultant proteïnuria, hypoalbuminaemia and azotaemia) and hypergammaglobulinaemia as was the case in this dog[14].

ACKNOWLEDGEMENTS

We would like to thank the staff of the Animal Hospital and Departments of Chemical Pathology and Radiology in the Faculty of Medicine at the Medical University of Southern Africa, and the staff of the Pathology Department at the Faculty of Veterinary Science, University of Pretoria, for their assistance in this case.

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FELINE INFECTIOUS DISEASES

NIELS C PEDERSEN

American Veterinary Publications, Inc, Goleta, California 1988. pp iii and 404, 122 figures. $64.95 (ISBN 0-939674-20-3)

Niels Pederson's book came at a most opportune time for veterinarians in the Republic of South Africa: feline practice is growing by the day, courses on feline disorders are being presented and cat foods are being promoted! Hopefully, this contribution will stimulate further investigation into feline disorders.

An introductory chapter is followed by 5 sections which deal with viral, bacterial, mycoplasmal, rickettsial, chlamydial, L-form induced, fungal and parasitic diseases. The latter section includes infestations with roundworms, flatworms, Acanthocephalons, arthropods as well as infections caused by protozoa. Diseases are discussed under the headings "etiologic agent, pathogenesis, clinical features, pathologic features, clinico-pathologic features, treatment and prevention, infection and immunity", as well as "animal and public health considerations". A list of references concludes the presentation on each disease.

I particularly enjoyed the section on viral diseases. The presentation on feline immunodeficiency virus infection with references to 1987 and 1988 publications, underscores the need for a sound laboratory back-up in the work-up and investigation of feline disorders. In the absence of such facilities, the book will definitely be of considerable help in the clinical diagnosis of feline diseases.

Despite the fact that very little has been published locally on cat diseases, most of what has been published on feline babesiosis, feline hepatozoonosis, encephalitozoonosis and eperythrozoonosis has been included in the text. This, in a way, highlights the dearth of information on cat diseases in this part of the world.

The book is an important contribution to veterinary science. The reproduction of the black-and-white photographs could, however, have been of a considerably better standard. I am also rather surprised at the relatively large number of typographical errors. Nevertheless, I have no reservation in strongly recommending this book to all practitioners, academicians and students who care about cats.

J VAN HEERDEN

CONCISE VETERINARY DICTIONARY

Robert S. Hine (General Editor)

Oxford University Press, Oxford OX26DP. 1988 pp 890, some illustrations. R75-00 (ISBN 0-19-854208-9)

Concise Veterinary Dictionary is more than just a dictionary: I found myself totally absorbed and eager to turn over each page. It does not only explain the meaning of a word, but provides the reader with an easy-to-understand concept of the significance of a word in veterinary context. "Ancylostoma" for example, is explained as a genus of parasitic nematodes which occurs in the small intestine of cats and dogs in tropical areas. The text then continues to briefly describe the clinical signs and pathophysiological events in infested animals as well as the life cycle and possible control of the parasite.

"Trenbolone acetate" serves as a good example of the detail in which generic names are eleborated upon: "a synthetic steroidal growth promoter with weaker androgenic activity than testosterone but similar anabolic properties. It is thought to act directly on muscle cells, causing a decrease in muscle protein turnover and consequently a net increase in protein synthesis. Plasma concentrations of the thyroid hormone thyroxine are reduced, lowering the metabolic rate to allow increased growth and better feed conversion efficiency. It is used as an implant in heifers and steers. In steers there is an increased effect if the drug is used in combination with an oestrogenic compound. Tissue residues are undetectable 60 days after implantation. Trenbolone acetate has been banned in the EEC as a growth promoter in food-producing animals because of consumer concern over residues in meat. It can still be used as an anabolic to promote protein synthesis, improve appetite, and increase calcium retention in various diseases (eg. chronic kidney disease, pregnancy toxaemia) and to improve fracture healing. Tradenames: Finaplix (implant); Finajet (injectable)."

The book, which obviously has been written with the British reader in mind, includes a wide range of topics: animal husbandry, biochemistry, diseases, disease-causing organisms, drugs, endocrinology, genetics, histology, meat inspection, physiology, surgical operations and instruments, toxicology etc.

The preface to the text states that the dictionary has been developed primarily for farmers, stockbreeders, agricultural and veterinary students, and veterinary assistants. The editor assumes that it will also prove to be useful to practitioners, pet owners, research workers in the pharmaceutical industry and in fields related to veterinary science. In my opinion, however, the dictionary should prove to be particularly useful to veterinarians and I have no hesitation in strongly recommending it to whoever has an interest in veterinary and related sciences.

J VAN HEERDEN

WILDLIFE DISEASES

OFFICE INTERNATIONAL DES EPIZOOTIES

Special issue of the OIE Scientific and Technical Review Volume 7, No 4, December 1988

The growing economic and cultural importance of wildlife in recent years can hardly be overlooked by the veterinary profession. This was why the Office International des Epizooties devoted a technical item of the 56th General Session in May 1988 to the study of diseases of wildlife transmissible to domestic animals

This subject is extremely complex. Interaction between wild and domestic animals can take many forms, wild species are themselves diverse and situations vary greatly from one country to another. Furthermore, the transmission of diseases does not occur in one direction only; if the health of domestic animals in contact with wild species quite rightly gives cause for concern, so should the impact of domestic animals on wildlife. The recent decimation of seal populations by canine distemper along European coastlines offers a timely reminder. Fortunately, many infections are highly specific and hence are not shared by different species; this substantially reduces the negative repercussions of existing interaction and suggests, too, that the specificity of some diagnostic methods should be re-examined.

The improvements in the economic and cultural status of wildlife have also encouraged the study of wildlife diseases as such and have led to reconsideration of the health aspects governing the marketing of wildlife products.

Without purporting to be exhaustive, this issue of the Scientific and Technical Review attempts to shed new light on the economic potential and utilisation of wildlife, interaction between wild and domestic animals, and the different aspects of disease control. It contains chapters on the status of wildlife diseases in various countries as well as chapters as wildlife diseases of particular importance in certain countries.

The following recommendations were made by the OIE International Committee at the 56th General session:

1. With respect to infectious wildlife diseases, methods of diagnosis and epidemiological investigations be improved and research intensified
2. The risks of transmitting diseases between wildlife and domestic animals and within wildlife populations be evaluated by appropriate methods.
3. Ecological and behavioural studies of wildlife be intensified with a view to developing methods for the management, control or prevention of transmissible diseases in wildlife, especially when introducing or reintroducing species or when there is an overpopulation of certain species.
4. Promotion of research into the sustainable production and marketing of wildlife products be undertaken.
5. That member countries take the necessary steps to extend their information systems on wildlife diseases and that the OIE Regional Commissions include wildlife diseases on the agendas of their conferences.
6. That member countries take steps to vaccinate wild animals against rabies, if possible after consultation with neighbouring countries.

GUIDE TO RUMINANT ANATOMY BASED ON THE DISSECTION OF THE GOAT

P D GARRETT

1st Iowa State University Press Edm., Ames, Iowa, 50010. 1988. PP VII and 102, illustrations 100. Price not mentioned. (ISBN 0-8138-0014-5)

This guide on dissection of the goat was compiled by the author for his anatomy department at Auburn University, Alabama. By the time his students embark on a 50 hours laboratory course on ruminant anatomy, they have already acquired a basic knowledge of mammalian anatomy - supposedly the dog and the horse. The author has, therefore, omitted elementary material such as the autonomic nervous system. Considering the effect of the autonomic system on the functional anatomy of the ruminant stomach, the reviewer finds this omission quite surprising.

The work purports to be a guide to ruminant anatomy. This is an overstatement. It is concerned mainly with identification of structures and indeed very little is offered in the field of systematic anatomy. The marked differences in the osteology of the sheep and goat are totally disregarded. Many aspects of the splanchnology of the 3 species are not mentioned at all, e.g. the vaginal part of the cervix in small ruminants, the postnatal development of the ruminant stomach, the exterior and interior of the forestomachs, the topography of the omasum, the blood supply to the brain. There is very little information on the paranasal sinuses and their communications.

Joints and ligaments are not important. The lymphatic system is limited to the identification of a few lymph nodes. One wonders how the newly qualified veterinarian will react when he is confronted with a bovine carcass during meat inspection. The description of the distal loop of the ascending colon is incomprehensible and wrongly illustrated.

The guide contains 100 schematic and faint illustrations of poor quality. They are not informative. There are two identical illustrations of a cranial view of the pelvic inlet - one for the cow (fig. 4-1) and one for the bovine (fig. 8-1). The dorsal and palmar/plantar views of the skeleton of the thoracic and pelvic limbs are depicted in reverse sequence to the captions. On P.67 *tuber ischium* and *tuber ischiadicum* are mentioned and ischium is spelt incorrectly.

Ruminant anatomy is fascinating, but few, if any, readers will be inspired by the account given in this guide. To relate what is written in this guide on the anatomy of the goat meaningfully to bovine anatomy as claimed by the author, is highly questionable. The reviewer cannot recommend this guide for use in the veterinary schools of this country.

J M W LE ROUX

FOOD IRRADIATION
A TECHNIQUE FOR PRESERVING AND IMPROVING THE SAFETY OF FOOD

1st Edn. Published by the World Health Organisation in collaboration with the Food and Agriculture Organisation of the United Nations 1988. 84 pages (available in English and French; Spanish in preparation) 3 figures and 3 annexures Price US $12,80 (ISBN 92 4 1542403) Order No. 1150302.

The book describes in a factual and objective manner the role of food irradiation as a technique for improving food safety and reducing food losses. It succeeds in explaining in non-technical language what the process involves, how it works and exactly what it achieves. The panel of international experts responsible for this review are aware of the negative public attitude towards food irradiation which is prevalent all over the world. They summarise scientific evidence in order to provide information and explanations to correct misconceptions that exist in an effort to help people to understand more clearly the role that food irradiation plays in securing an adequate, wholesome and dependable food supply.

The book reminds the reader of traditional methods of food preservation, highlighting advantages and disadvantages, and comparing them to the process of food irradiation. The third chapter provides useful background information and elaborates on the in-depth studies undertaken to date on the effects of irradiation on food and the safety and quality of irradiated food. The reader is then introduced to the methods of food irradiation and the results achieved in some 34 countries. Annexure 1 details which countries have approved irradiated food for human consumption, giving details of the product, the purpose of irradiation and the permitted dose. Of interest to South African readers is the review listing various problems that need to be addressed in undeveloped tropical countries and also in developed countries when considering the implementation of this process. Legislation and control of food irradiation is touched on in the text and elaborated on in 2 annexures:

X The Codex General Standard for irradiated foods
X Recommended International Code of Practice for the operation of irradiation facilities used for the treatment of food.

In the final chapter, 16 questions concerning food irradiation that are frequently raised by consumers and consumer groups, are answered. The technical accuracy of the book has been checked by the Food Preservation Section, Joint FAO/IAEA Division, at the Atomic Energy Agency, Vienna and the WHO units concerned with Radiation Medicine and Prevention of Environmental Pollution, Geneva, Switzerland..

The book is well presented and technically sound, but obviously cannot cover too extensively the scientific and technical literature on one of the most emotional health issues of the 1980's. The reader who wants more detailed information is provided with a comprehensive bibliography, which covers most of the subjects dealt with and provides a useful guide to further reading. Disappointing is the omission from the extensive list of booksellers around the globe of a South African supplier of World Health Organisation publications.

C M VEARY

ENROFLOXACIN: A NEW ANTIMICROBIAL AGENT*

J. SCHRODER**

ABSTRACT

A review is given of available literature on a new antimicrobial agent, enrofloxacin. The chemical is a quinolone carboxylic acid derivative, and has a broad spectrum of activity against Gram-positive and Gram-negative bacteria as well as against *Mycoplasma* spp. in various animals. Several formulations and routes of administration have been tested, and equally good absorption and efficacy have been demonstrated after oral and parenteral administration. In South Africa, a 10% solution for use in the drinking water of poultry, has been registered, while different formulations for other species are still under development.

Key words: Enrofloxacin; antimicrobial; review

Schröder J. Enrofloxacin: A new antimicrobial agent. *Journal of the South African Veterinary Association* **(1989) 60 No. 2, 125-127 (En.) Bayer (SA) Animal health Division, P.O. Box 143, 1600 Isando, Republic of South Africa.**

INTRODUCTION

Enrofloxacin (Baytril, Bayer) was first synthesised in 1983, and belongs to the group of quinolone carboxylic acid derivatives[26]. Following the establishment of its safety (good therapeutic index[6], lack of teratogenicity or mutagenicity[1]), clinical testing of the compound was completed in a remarkably short time. For this purpose, various formulations were used against a range of natural and artificial bacterial infections in different organs, in a variety of animal species.

This review reports on the antimicrobial spectrum, historical development, applications, pharmacokinetics, mode of action, and possible advantage of enrofloxacin as a new veterinary drug.

HISTORICAL DEVELOPMENT

Enrofloxacin is one of the chemicals developed from nalidixic acid[1]. The antimicrobial properties have been enhanced and adverse effects reduced through various gradual modifications to the molecule. These include several changes to the A ring (substitution of the N atom by CH, introduction of an F atom, and substitution of the methyl group by piperazinyl) and variation of the substituent on the N atom in the B ring (Fig. 1).

ANTIMICROBIAL SPECTRUM

The minimum inhibitory concentrations (MIC) of enrofloxacin were determined against more than 100 different bacterial strains by several independent investigators[9 26 32]. Most of the tests were done in ISO-sensitest broth with a seeding density of 100 000 organisms ml^{-1}, and incubation at 37° C for 24 h. Organisms such as *Mycoplasma, Haemophilus,* and *Clostridium* had their particular cultivation requirements catered to. Incubating the bacterial cultures under anaerobic or aerobic conditions did not affect the in vitro efficacy of enrofloxacin, nor did variation in pH of the culture medium ranging from 6 to 8.

As can be seen in Table 1, the antibacterial efficacy of enrofloxacin covers a broad spectrum of Gram-positive and Gram-negative bacteria, as well as the clinically important *Mycoplasma* spp. The mean MIC values range from 0,008 to 4 $\mu g\ ml^{-1}$.

The in vitro efficacy of enrofloxacin was compared with a number of antibiotics and antibacterial compounds such as ampicillin, chloramphenicol, gentamicin, penicillin G, tetracycline, and trimethoprim/sulphonamide against a number of bacteria (eg. *Escherichia, Klebsiella, Salmonella, Pasteurella,* and *Staphylococcus*). Enrofloxacin was superior in efficacy against the Gram-negtive organisms tested, whereas the MIC values against Gram-positive bacteria fell within the range of ampicillin, oxacillin, penicillin G, and gentamicin.

Table 1: **The antibacterial spectrum of enrofloxacin (Baytril®: Bayer)**

Organism	Strains	MIC range ($\mu g\ ml^{-1}$)	MIC mean
E.coli	178	<0,01-0,5	0,06
Klebsiella spp.	48	<0,03-0,5	0,06
Salmonella spp.	155	0,003-0,5	0,03
Proteus spp.	55	0,03-0,5	0,25
Serratia marcescens	20	0,01-1,0	0,12
Citrobacter sp.	3	0,25-0,5	0,25
Yersinia spp.	5	0,01-0,04	0,01
Campylobacter spp.	31	0,03-0,25	0,25
Pseudomonas aeruginosa	41	0,25-2,0	0,75
Brucella canis	3	0,1-0,25	0,25
Bordetella bronchiseptica	31	0,1-4,0	0,5
Moraxella bovis	5	0,03-0,05	0,03
Haemophilus spp.	23	0,02-0,5	0,02
Pasteurella multocida	78	<0,001-0,12	0,008
Pasteurella haemolytica	52	0,008-0,12	0,06
Vibrio parahaemolyticus	25	<0,01-0,4	0,2
Treponema hyodysenteriae	5	4,0	4,0
Bacillus cereus	48	0,06-0,5	0,25
Staphylococcus aureus	135	0,03-1,0	0,12
Staphylococcus hyicus	12	0,01-0,4	0,12
Streptococcus spp.	61	0,06-4,0	0,75
Corynebacterium pyogenes	29	0,06-4,0	0,75
Listeria monocytogenes	5	1,0-2,0	1,75
Erysipelothrix spp.	6	0,06-0,1	0,06
Mycoplasma spp.	92	0,01-1,0	0,25
Actinobacillus spp.	8	0,01-0,3	0,03
Bacteroides spp.	25	0,8-12,5	1,6
Clostridium perfringens	33	0,2-2,0	0,5

The mean MIC values of enrofloxacin were 20 - 30% of those of flumequine, another quinolone carboxylic acid derivative. Flumequine is ineffective against streptococci and mycoplasmas, but from Table 1 it is evident that even enrofloxacin experiences some difficulty in inhibiting *Streptococcus* spp.

* Paper delivered at the Biennial Congress of the South African Veterinary Association, Pietermaritzburg, July 1988

** Bayer (SA) Animal Health Division, P.O. Box 143, 1600 Isando, Republic of South Africa

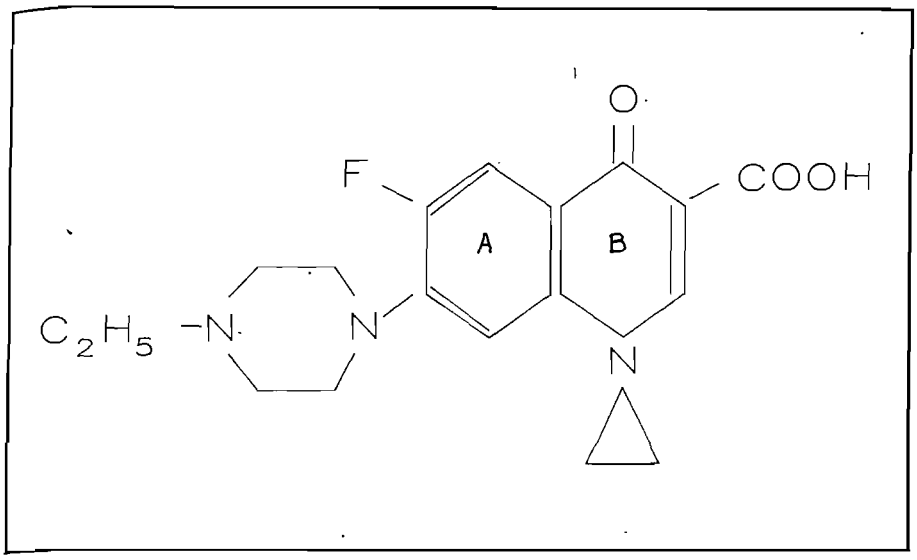

Fig. 1: **The structural formula of enrofloxacin**

APPLICATIONS

The efficacy of enrofloxacin was evaluated in clinical trials involving more than 1 100 calves and 4 000 pigs suffering from natural infections of the respiratory and digestive organs, and in experimental disease models[6 14]. The therapeutic effecf in puerperal diseases of the pig (the so-called MMA syndrome) was also studied[21].

In calves, 3 - 5 daily treatments with enrofloxacin at 2,5 mg kg^{-1} (oral or parenteral administration) were highly effective against natural and artificially induced colibacillosis[6 23]. In treated calves with a slight diarrhoea, treatment also prevented the occurrence of intercurrent diseases, such as bronchopneumonia and omphalitis, which occurred in the untreated controls[6 24]. In newborn, artificially infected calves, the most successful regimen[17] was an initial intramuscular injection at 1 mg kg^{-1}, followed by 2 oral treafments af 2,5 mg kg^{-1} day^{-1}.

Clinically evident *Salmonella* infections in calves were treated successfully by oral administration at 2,5 mg kg^{-1} d^{-1} for 5 d, but the dosage may have to be increased[5 6] to 5 mg kg^{-1}.

Enrofloxacin at 2,5 mg kg^{-1} d^{-1} for 3 to 5 d (the duration of treatment should be adapted to the clinical response[5]) was highly effective in calves against simultaneous experimental respiratory infections with *Pasteurella haemolytica* and *Mycoplasma bovis*. Natural infections with *Pasteurella multocida* and *Pasteurella haemolytica* in calves were used to prove that oral treatment at 2,5 mg kg^{-1} d^{-1} for 5 to 8 d was effective. Nothing could be gained by increasing the dosage[6 22 31].

The efficacy of enrofloxacin in pigs was tested in cases of bronchopneumonia, enzootic pneumonia, and artificial infections with *Mycoplasma hyopneumoniae*, *Salmonella derby*[6 20], *Escherichia coli*[2 3 11] and *Haemophilus pleuropneumoniae*[30 33]. Three intramuscular injections at 2,5 mg kg^{-1} against the respiratory conditions and colibacillosis[3 6], feed medicated at 200 ppm (salmonellosis[6]) or 50 ppm (colibacillosis[3]) and oral treatment at 2,5 mg kg^{-1} against colibacillosis[11] proved to be highly effective.

Experimental infections with *Mycoplasma* spp., *E. coli*, *Haemophilus paragallinarum*, *Salmonella* spp., *Pasteurella multocida*, and *Erysipelothrix rhusiopathiae* were used to study the effect of enrofloxacin in broiler chickens and turkeys[7 13 15 16]. Treatment via the drinking water at 50 mg ℓ^{-1} (ppm) for 3 d was found to be effective. Subcutaneous injections at 5 mg kg^{-1} prevented outbreaks of disease in turkey poults after artificial infections with *Salmonella arizonae*[7]. Enrofloxacin at a concentration of 500 mg ℓ^{-1} was also shown to be effective as a turkey egg dip against *Salmonella arizonae*[12].

Dogs and cats with infections of the digestive, respiratory, and urogenital tracts, infections of the skin and external auditory canal and wounds, were treated successfully with enrofloxacin in tablet form or by subcutaneous injection at 5 mg kg^{-1} d^{-1} for 5 to 10 d[8 10].

Various *Mycoplasma* spp. are implicated in balanoposthitis and ulcerative vulvo-vaginitis in sheep[32]. Although the in vitro efficacy was good, treatment of infected sheep was disappointing, probably because an appropriate vaginal formulation for sheep was not available. Intramuscular injections at 5 to 10 mg kg^{-1} had no effect, while intravaginal instillation of 2ml of a 5% solution had a limited effect.

PHARMACOKINETICS

Different dosages and routes of administration were used to determine the levels and duration of serum and tissue concentrations of enrofloxacin in cattle, swine, dogs, cats, broiler chickens and turkeys[4 18 25 27 28 29]. Calves were given 2,5 or 5 mg kg^{-1} either orally, or by intravenous, intramuscular or subcutaneous injection. Pigs received 2,5 mg kg^{-1} orally or by intramuscular injection. Cats and dogs were given 2,5 or 5 mg kg^{-1} enrofloxacin by subcutaneous injection, or 5 mg kg^{-1} orally. The poultry received 2,5 mg kg^{-1} by subcutaneous injection or orally, or drinking water medicated at 25, 50, or 100 mg ℓ^{-1} for 14 d, or medicated feed containing 50 or 200 mg kg^{-1} enrofloxacin, also for 14 d.

Enrofloxacin levels in serum, other body fluids, and tissues were determined microbiologically in a diffusion test on ISO-sensitest agar against a sensitive *E.coli* strain.

Maximum concentrations were generally reached within 0,5 to 2 h, with the injections (subcutaneous or intramuscular) which acted more rapidly than oral administration. This latter observation does not hold true for pre-ruminant calves, where administration to the base of the tongue resulted in a more rapid attainment of peak plasma concentrations.

MODE OF ACTION

The quinolone carboxylic acid derivatives inhibit the bacterial enzyme DNA-gyrase[26]. The effect of this mode of action was visualised by sequential microphotography of treated cultures of 2 bacteria, *Staph.aureus* and *E.coli*[19]. The conclusion was that the enrofloxacin inhibited cell division (that no septa formed), and that the cells enlarged and eventually lysed.

In addition, an electron microscope was used[34] to photograph cultures of *Staph.aureus* and *E.coli* 2 h after adding enrofloxacin at concentrations of 0,025 -2,0 μg ml^{-1}. The earliest structural changes seen were a peripheral loosening of the bacterial plasma and elongation of the Gram-negative organisms, and at increasing concentrations, lysis and rupture of cell membranes. In staphylococci, the damage starts with deformation of the septa, and leads to deformation of the entire bacterial cell, and finally lysis.

SAFETY

Enrofloxacin at dosages in excess of 10 times the therapeutic level did not elicit significant changes in the vital parameters of laboratory animals[1]. It is neither anti-allergic nor pseudo-allergic, and has no influence on the cardiovascular or central nervous systems (except for stimulation of spontaneous motility at dosages exceeding 100 mg kg^{-1}). The no-effect level in rats and dogs was found to be 2 000 mg enrofloxacin per kg feed in a 13-week sub-chronic toxicity study. Enrofloxacin is neither embryotoxic, teratogenic, nor mutagenic.

Primary degenerative damage to the cartilage of weight bearing jounts, a lesion characteristic for this class of compounds, was seen in Beagle pups treated at 30 - 60 mg kg^{-1} for 14 d[8]. Both the dosage and duration of this treatment were in excess of those generally recommended for therapy. The use of enrofloxacin in dogs under the age of 1 year is nevertheless contra-indicated in view of these findings.

ADVANTAGES

Enrofloxacin posesses the following properties which, although not unique, in combination make it a desirable antimicrobial compound in the veterinarian's armamentarium: a. broad spectrum of activity against Gram-negative and Gram-positive bacteria and mycoplasmas, b. bactericidal and mycoplasmicidal activity at concentrations mostly only twice the minimum inhibitory concentrations, c. efficacy against organisms showing resistance against antibacterial substances such as β-lactam antibiotics, aminoglycosides, tetracyclines, and folic acid antagonists, d. high bioavailability after oral or parenteral administration, e. good therapeutic index, and important in chickens, f. no incompatibility with known anticoccidials.

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