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TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

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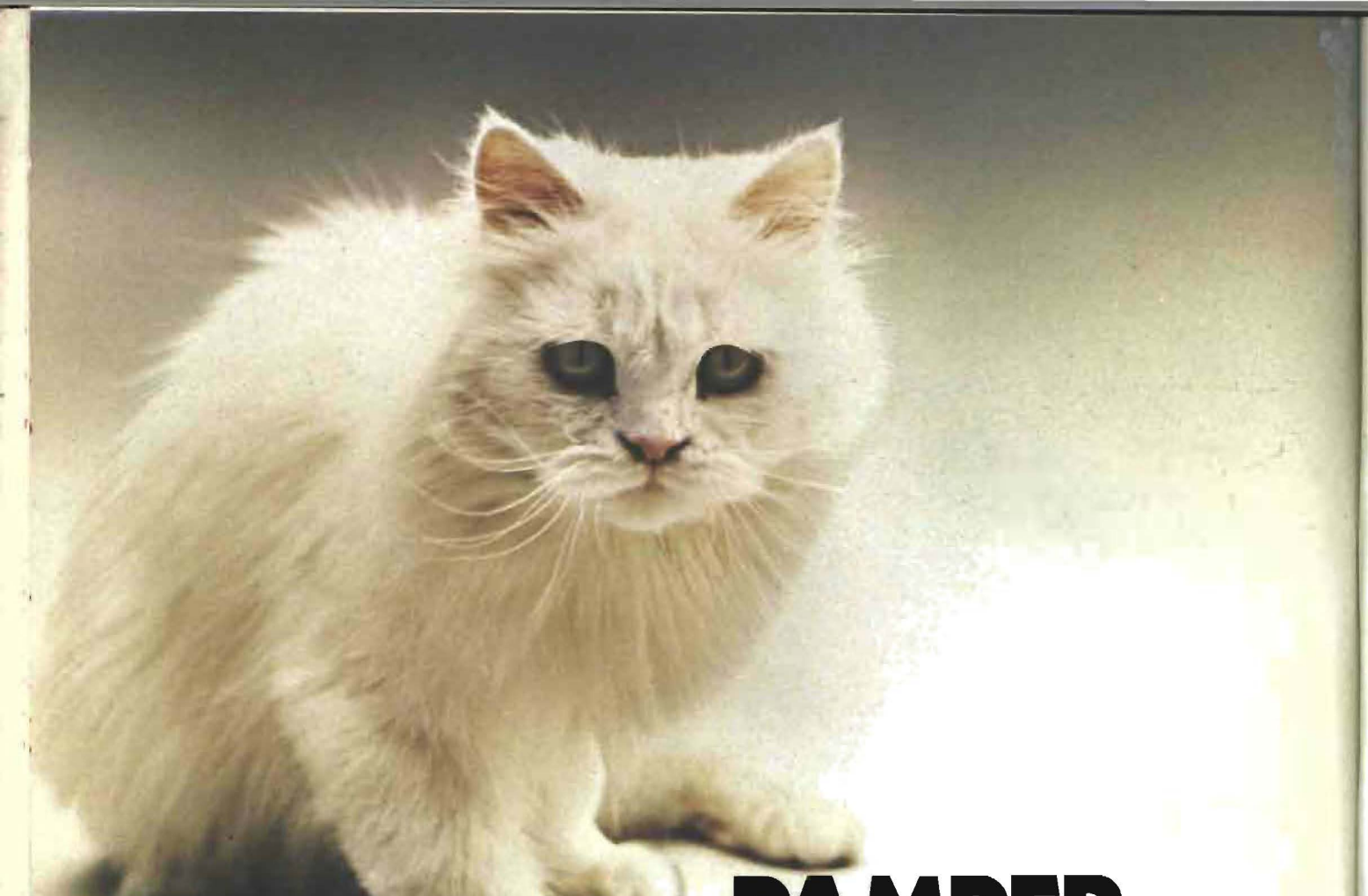

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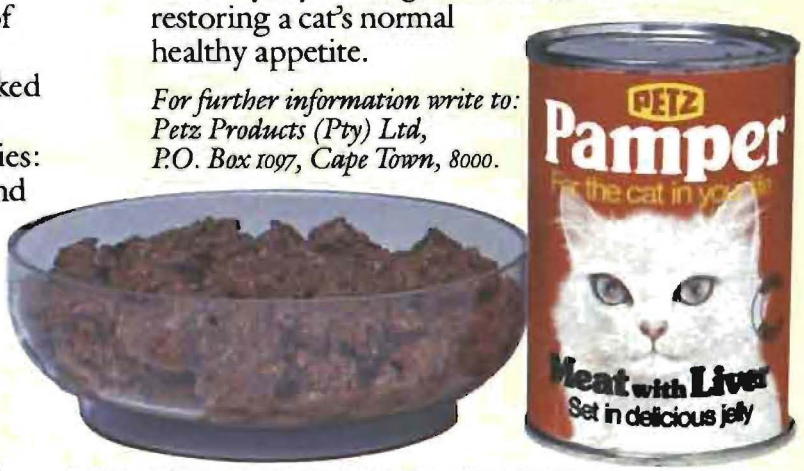

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JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

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CONTRIBUTIONS – The Editor will consider contributions of veterinary interest. Double-spaced, carefully revised, typewritten manuscripts should be submitted in triplicate (original plus first two copies). Layout and references should be in the style of this number. REFERENCES should not exceed 20 in number unless approved by the Editor. The number of figures and tables may be limited at the Editor's discretion unless the author contributes to the cost of reproduction. This applies particularly to reproductions in colour.

TABLES and FIGURES should be in widths of 85 mm, or 176 mm, or in sizes of 263 × 176 mm, or reducible thereto. Only the International Metric System (SI) is used in this Journal and contributors must ensure that fluid volume, length, mass, time, amount of substance, etc. are indicated in the correct SI unit. Time is expressed as: a (year), week, d (days), h (hours), min (minutes) and s (seconds). For further information refer to M33a (SABS, P/Bag X191, Pretoria). REPRINTS should be ordered upon confirmation of publication. The senior author receives 25 reprints of each article free.

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Die TYDSKRIF is die offisiële mondstuk en eiendom en word gepubliseer deur die Suid-Afrikaanse Veterinêre Vereniging. Dit verskyn kwartaalliks en word aan sake van algemene veeartsenykundige belang gewy. Bydraers tot hierdie Tydskrif maak hul stellings en lug hul menings slegs op eie verantwoordelikheid; sodanige stellings word nie noodwendig deur die Redaksiekomitee onderskryf nie en die menings gee nie noodwendig die Komitee se menings weer nie. Kopiereg word op al die letterkundige inhoud van die Tydskrif voorbehou.

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GUIDE FOR AUTHORS WHO WISH TO PUBLISH IN THE JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

1. The Journal is the official organ of the South African Veterinary Association (SAVA), and its object is the furtherance of the veterinary profession and of veterinary science in the Republic of South Africa by the publication primarily of articles on original and/or clinical investigation dealing with veterinary science in general. Reviews and other types of articles will be published after having been judged on their merits, as will be preliminary communications or notes. The whole of the literary contents of the Journal is copyright.
2. Articles written by members of the SAVA will have precedence over those written by non-members. The statements made and opinions expressed by contributors are their responsibility only; such statements are not necessarily endorsed by the Editorial Committee (EDCO), nor do the opinions reflect those of the Committee.
3. Submission of a manuscript will be regarded as tacit declaration by the author that the same material has not been accepted for publication elsewhere, except possibly as a preliminary report or as a privately printed dissertation, and if accepted it shall not be published in any other journal without the Editor's permission.
4. As far as possible the date of receipt of an article will determine the order of publication, but this will also depend on the amount of corrections necessitating the article having to be returned to the author. All contributions will be subject to editing by the EDCO.
5. Contributions must be in Oxford English (use the Concise Oxford Dictionary and Fowler's Modern English Usage – i.e. not American English) or Afrikaans. If in Afrikaans, it is obligatory that the abstract be in English.

6. Typing of contributions

Articles should be typed in double-spacing, on one side only of A4 paper with a 30mm margin on the left side. **The original copy and 2 good copies or photocopies should be submitted, each with a complete set of figures and tables.** Manuscripts (MS) should be carefully revised before submission.

7. Lay-out of articles

This will depend on the type of article, but most articles on original investigations should be divided into the following sections and order:

TITLE
NAME(S) OF AUTHOR(S)
ABSTRACT
INTRODUCTION
MATERIALS AND METHODS
RESULTS
DISCUSSION
ACKNOWLEDGEMENTS
REFERENCES

Deviations from the above order are, however, permissible and authors are allowed some flexibility and individuality if this is warranted.

Major headings such as those above should be typed in **capitals** in the **centre** of the page (and are **NOT underlined**).

The following categories of contributions are in general use:

EDITORIAL(S) — VAN DIE REDAKSIE

ADDRESS(ES) — VOORDRAG(TE)
Opening addresses, presidential addresses, addresses delivered to audiences on general and/or scientific matters of veterinary interest

ARTICLE(S) — ARTIKEL(S)
Original reports of research, field investigations, etc., **not** previously published elsewhere

REVIEW(S) — OORSIG(TE)
Comprehensive reviews of the literature dealing with a specific subject

RESEARCH NOTE(S) — NAVORSINGSNOTA(S)
(See 29)

SHORT COMMUNICATION(S) — KORT BERIG(TE)
(See 29)

CLINICAL COMMUNICATION(S) — KLINIESE BERIG(TE)
(See 29)

CASE REPORT(S) — GEVALVERSLAG(E)
Short, preferably illustrated, informative reports of interesting and/or unusual cases from the field or clinical practice. Must be original (see 29)

CONTINUED EDUCATION — VOORTGESETTE OPLEIDING

INFORMATION — INLIGTING
General "snippets" of information of veterinary or related interest – must be brief

FEATURE PAGE — TREFFERBLAD
Single page, brief, preferably in English and Afrikaans, must be illustrated (see 28)

OUT OF THE PAST — UIT DIE VERLEDE
Contributions of historical interest

TO THE EDITOR — AAN DIE REDAKSIE
Letters to the Editor (see 27)

BOOK REVIEWS — BOEKRESENSIES
(See 26)

FACULTY NEWS — FAKULTEITSNUUS
New graduates, awards, prizes, etc.

ASSOCIATION NEWS — VERENIGINGSNUUS
Awards, information, newly elected presidents and vice-presidents plus photographs and introduction, etc.

8. Title of article, case report, etc.

This should be as **brief** as possible and should not contain abbreviations of any nature, e.g. CSF, or abbreviations following a word or phrase, e.g. cerebrospinal fluid (CSF). Titles such as "AN OUTBREAK OF PARAMPHISTOMUM MICROBOTHRIUM FISCHOEDER 1901 INFESTATION IN THE HIPPOPOTAMUS (HIPPOPOTAMUS AMPHIBIUS LINNAEUS 1758)" should be shortened to "AN OUTBREAK OF PARAPHISTOMIASIS IN THE HIPPOPOTAMUS" unless of specific taxonomical significance.

Have the title typed in capitals. There is no full stop after the title or any heading.

9. Names and addresses of authors

9.1 Names of authors should be typed in capital letters in the centre of the page beneath the title. Full stops should be used after initials.

9.2 At least one first name of an authoress is given.

9.3 "and" (in small letters) and not the ampersand (&) is used between 2 authors, e.g.:

J.M.M. BROWN* and ANNA M. VERSTER**

9.4 If an article has only **ONE author,** his **postal address** shall appear **BOTH** in the **ABSTRACT** and as a **FOOTNOTE** which shall be correlated with his name by means of an asterisk.

The postal address of a member of a Faculty will be, e.g. Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa; and that of a member of the Veterinary Research Institute, e.g. Section of Food Hygiene, Veterinary Research Institute, P.O. Box 12502, 0110 Onderstepoort, Republic of South Africa.

N.B. The position of the postal code and the comma preceding but not following it.

9.5 Should there be **MORE THAN ONE author:**

1. The **postal address** of the **first** author shall appear **both in the abstract** (see 9.4 above) and **as a footnote** which shall be correlated with his name by means of an asterisk, and
2. Only the institute, department, organization or work situation of the co-authors, and **NOT** their full postal addresses, shall be correlated with their names by means of asterisks and footnotes on the title page. For example, in the case of an article by:

J. BURGER*, G. BROWN**, P. YSSEL**, S. VAN NIEKERK***, I. SMIT† and JANE CITIZEN‡‡

the footnote on the title page might read:

*Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.
**Private Practitioners, Randburg.
***Veterinary Research Institute, Onderstepoort.
†Faculty of Agriculture, University of Stellenbosch.
‡‡Ciba-Geigy (Pty) Ltd, Isando.

10. Abstract

This is the most frequently read part of the article. It appears in abstracting journals – some of which print direct photostats of it.

10.1 The typewritten format of the abstract shall be as follows (note the use of capital letters, the abbreviation (En) for "written in English", (Afrik) "written in Afrikaans", punctuation, underlining, brackets, omissions where

the volume number, issue number and page numbers will be placed at a later stage by the EDCO, and the addition of index key words for computerization which are placed at the end of the abstract). *Example:*

ABSTRACT: Nichol T.K.; Fregin G.F.; Gerber N.H.; Jones K. An outbreak of anthrax in kudus in the Kruger National Park. Journal of the South African Veterinary Association (1978) ..... No. ..... (En) Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

Sporadic outbreaks of anthrax in kudus .....
.....
..... during droughts.

Key words: Anthrax, Bacillus anthracis, kudu, national park, zoonosis.

(*Note:* The Editor will later indicate that the title and the volume number must be printed in bold type. The Journal name can be underlined in the original MS because it will be italicized.)

10.2 Abbreviations e.g. CSF are **NOT permissible** in the abstract except under exceptional circumstances – see also section on "Abbreviations" below.
If abbreviations must be used in the abstract, the first time that they do appear, their meanings should be clearly stated, e.g. cerebrospinal fluid (CSF). **These should then be repeated IN TOTO in the body of the text,** i.e. cerebrospinal fluid (CSF).
Abbreviations of generic names of e.g. a bacterium should not be used the first time they appear in the abstract, unless they have appeared in full in the title. (See also "Abreviations" below.)

10.3 The "body" of the abstract should be meaningful and free from "vague unhelpfulness". It should state briefly and precisely what was done, what emerged and what conclusions were reached; e.g. it is not sufficient to state that "The effect of vaccination with blackquarter vaccine in cattle was studied" but rather that "Twenty two-year-old susceptible cattle were immunized against blackquarter. Three doses of formal-toxoid administered at intervals of 21 days produced an immunity 10 times better than one dose".

10.4 It is obligatory that the abstracts of Afrikaans articles should be written in English and desirable that they be fairly comprehensive.

10.5 Key words should be given at the end of the abstract. There should not be more than 10 and their choice depends on the subject of the paper. Libraries require them for computerization purposes.

10.6 An abstract must form part of each article, case report, research note, clinical and short communications, and review-type and historical contributions.

11. Materials and Methods

Under this heading it should be stated precisely (and as concisely as possible) what was used and done in the experiment – so that anyone wishing to repeat the experiment can do so.

12. Abbreviations and Symbols (See also Addendum)

Abbreviations such as those for formal-toxoid (FT), cerebrospinal fluid (CSF), etc., should be avoided if possible. They should be used only when the frequent use of a rather long and cumbersome term makes an abbreviation an aid to easy reading. (The operative words here are **frequent, long** and **cumbersome.** It is absurd to use an abbreviation if a term is used only 2 or 3 times in an article.) If they are used, their meanings should be clearly stated when first introduced, and they should be written as combinations of capital letters without spaces and full stops.

They should be redefined in the body of the article if used in the abstract, and should never be used in the title.

After scientific names (generic and specific) of e.g. a bacterium have been used the first time in full, the generic name may be abbreviated if used again. This, however, excludes the use of such names in the title or abstract – i.e. if Bacillus anthracis is used in the title and/or abstract, it must be written out in full the first time that it is used in the text – Bacillus anthracis. (See also 18.)

Other common abbreviations (note the presence or absence of full stops and the absence of italics – i.e. underlining in typewritten manuscripts):

et al.	p page
i.e.	pp pages
cf.	ad lib.
etc.	ed. editor(s)
% for per cent (leave a gap between sign and numeral)	edn edition
N.B.	Fig. (singlular and plural)
S.G.	Smith & Jones (ampersand (&) is used only in text).
sp.	1–6 (use dash – and not "to" unless it is preceded by a preposition when the "to"
spp.	should be written, e.g. "The farmer dosed 5–10 mg of copper sulphate. There
var.	were from 7 to 11 animals in each group").

SD
SE
Mr Mrs
Dr Prof.
No. (number)

mg/kg (use the solidus (line) / for "per" when it is flanked on **both** sides by metric and/or SI symbols);

Examples:
30 km/d (and **not** 30 km/day – if you wish to write "day" out in full, the correct version is: 30 km per day. "km" and "d" are metric or SI symbols but "day" is not).

SI (Système International d'Unités) symbols must be used (this includes the metric system in its most modern form). These symbols as well as certain other abbreviations are given in the **Addendum** to this Guide to Authors.

13. Numerals

A sentence should never commence with a numeral. The number should either be spelt out or the sentence recast.

Use a numeral for every number used above one. The numeral "one" should be spelt out if it appears alone in the text and not amongst a series of other numbers. If it is part of a unit of measurement, the numeral 1 should be used.

Examples:
(1) The farmer took one cow to market, but 3 piglets and 25 horses stayed at home.
(2) From 1 to 6 cattle were in each group ("to" is used here and not a dash because 1 to 6 is preceded by a preposition; otherwise use a dash between two numbers to denote a consecutive series, e.g. "In Tables 1–7 and Fig. 13–20 are shown the . . .").
(3) 1 h, 1 min, 1 week, 1 month, 1 g, 1 l, Day 1, Table 1, Fig. 1, Horse 1, Stable 1, etc. (N.B. Notice the use of capital letters in Day 1, Stable 1, etc.).

14. Fractions

When not using mathematical formulae, dilutions, etc., fractions such as a quarter, a half, a third, an eighth should be written out in full, e.g. "Of those present, half had received inoculations." Cumbersomeness should, however, be avoided – 1/190 is acceptable but not one one hundred and ninetieth.

15. Underlining

The ONLY words to be underlined in a typewritten manuscript are those which will appear in italics in the printed article. Headings such as MATERIALS AND METHODS, legends to figures, etc, should therefore NOT be underlined.

16. Italics

Only words to be italicized are to be underlined in typewritten manuscripts.

The names of phyla, classes, orders and families of plants, etc., are not italicized but genera, subgenera, species, subspecies and varieties are. The abbreviation var. (= varietas) appearing between the name of a species and that of a variety must not be italicized, e.g. Hyalomma marginatum var. olenevi Schulze & Schottke, 1930.

Generic names used adjectivally (e.g. salmonella type, staphylococcus toxins) and common or Anglicized names (e.g. streptoccoccus, corynebacteria, mycobacteria, mycoplasmas, anaplasmas) are not italicized.

Names of journals or text books when referred to in the body of an article should be italicized, e.g. "In a leading article in the Veterinary Record it was . . .". "Smith quoted directly from the book Veterinary Pathology to illustrate . . ."

The following should **NOT** be italicized:

in vitro	per os	et al.	corpus luteum	corpus luteum gravidum atreticum
in vivo	per rectum	i.e.	tunica propria	tunica muscularis
in utero	vide supra	e.g.	muscularis mucosa	post partum
in situ	vide infra	cf.	vice versa	
in toto		etc.	post mortem	
		ad lib.		

N.B. When using the symbol l for litre, the l must always be italicized otherwise it might be mistaken for 1 (one). In print it will look like this: *ℓ*. If, however, the symbol for millilitre (ml) is used, do not underline the l because no confusion can arise in this case.

17. Capitals

Where possible, the use of capital letters to start words should be avoided except where spelling and grammatical rules dictate otherwise, e.g.

blue wildebeest
African grey parrot
African oriole
bluetongue
Rift Valley fever

However, names of breeds of animals should commence with a capital letter, e.g. Fox Terrier, Bulldog, Thoroughbred, Merino.

When referring to a Table or Fig. in the text, these words should start with a capital letter, as also when referring to a specific day, e.g. Day 1, or specific object or place, e.g. Group 6, Cow 1, Camp 2, Stable 5.

Names of proprietary preparations should commence with a capital letter, e.g. Chloramex but the active principle chloramphenicol is written without the capital. (See also paragraph 19 below.)

The correct manner to write surnames which include a particle (such as Van Rensburg) is as follows:

"Prof. Du Toit, Mr Le Roux and Dr Van der Merwe were present."
"Prof. Philip du Toit, Mr Pik le Roux and Dr J. van der Merwe did not attend."
"Van Rensburg, Von Backstrom and Le Riche tied for first place."

The first letters of the particles Du, Le, Van, Von, etc. are thus written as capitals unless preceded by a name, a nickname or an initial.

18. Nomenclature

Recognized names of organisms should be used. If there is any possibility of confusion or if any organism has recently received a new name, a synonym or the old name should be mentioned in parenthesis, e.g. Clostridium feseri (syn. Clostridium chauvoei) the first time that it appears in the text.

The binomial name of an organism (e.g. Pasteurella multocida) should be written out in full when it is first used, thereafter the generic name may be abbreviated (e.g. P. multocida) in most cases – but see below in this paragraph. This is also the case when the names of a series of organisms of the same genus are used for the first time, e.g. "Clostridium botulinum type B, Clostridium haemolyticum and Clostridium chauvoei were not present in the contaminated material but Corynebacterium renale, Corynebacterium ovis and Coxiella burnetii occurred in large numbers." If the abbreviation of generic names could lead to confusion e.g. Taenia ovis (abbreviated to T. ovis) and Trichuris ovis (abbreviated to T. ovis), the names must not be abbreviated. The complete binomial names shall appear in full throughout the article.

In taxonomic articles the full name of an organism, plant, etc., together with the name of the author(s) and the year of publication should be given in the text when used for the first time, e.g. Hippopotamus amphibius Linnaeus 1758.

Use arthropod instead of arthropoda, etc.

19. Names of chemical substances, drugs and apparatus

These should conform to current chemical practice; formulas may be used for the commoner compounds. If a proprietary preparation or remedy is mentioned in the text, the generic name(s) of the substance(s) must be used, followed by the trade name and the name of the manufacturer in brackets.

Examples: "The animal was treated with sulphadoxine and trimethroprim (Borgal V, Hoechst)."

"The dog was dosed with a bronchodilator containing ephedrine sulphate, theophylin monohydrate and phenobarbitone (Franol, Winthrop Lab.)." Greater details can, if necessary, be given of the dosage rate and strength of the preparation.

If equipment has to be identified, mention the name of the apparatus followed by the specific make and model in brackets.

Example: "A spectrophotometer (Perken-Elmer Model B6) was used for analysis".

20. Use of first person

This may be used to a limited extent. Avoid excessive use of it.

21. Punctuation

Correct punctuation should be used.

No full stops should be used after headings or subheadings, e.g. MATERIALS AND METHODS

Notice the use of full stops in abbreviations such as e.g., etc., et al., and so on (see also 12).

22. References

A number system is used (see 22.3).

22.1 References in the text

When 3 or more authors have written an article and you wish to mention the name of the senior author in the text, use the abbreviation et al. right from the start, e.g. "Adams et al.[5] stated that . . .", but if only 2 authors are involved both names are mentioned, e.g. ". . . as was described by King & Johnson[7]."

If names of authors are referred to, use the ampersand (&) to indicate co-authors and "and" to indicate different publications, e.g.

Jones & Smith[4] found . . .
Jones[4] and Smith[10] found . . .

Adams et al.[1], Jones & Smith[4] and Robinson[8] found . . .
When 2 authors of the same name are cited in the text, the initials should be included, e.g. J.A. Smith[4] and S.A. Smith[5] found . . .

Please note:
The use of a dash and the placing of the puntuation marks if they are used after a reference number in the text, e.g.

. . . . . dipping of animals[3–5].
. . . . . dipping of ewes[4 7–11], and dosing of wethers[4 6 9 10].
Bird et al.[1] decided that . . . . . .

(Notice the absence of commas between reference numbers.)

(AND NOT

. . . . . dipping of animals.[3,4,5]
. . . . . dipping of ewes,[4,7,8,9,10,11] and dosing of wethers.[4,6,9,10]
Bird et al[1]. decided that . . . . . .)

If you are having an article typed, it usually pays dividends if you give the typist a copy of the journal in which the article will eventually appear to serve as an example.

When referring to publications of different authors or groups of authors, their names should be given in chronological order, e.g. "Bird & Tatlock[4], Van der Westhuizen[8], Alberts et al.[1] and Mullins & Smith[5] decided to investigate . . ." (4 might be 1930, 8 – 1935, 1 – 1940 and 5 – 1945, etc.)

22.2 Citations in the text

When a paper was not seen and is merely referred to on authority of another publication, only the name of the author who cited the work of the other should be included in the list of references. The name of the author who reported the original work (i.e. the unseen report) should NOT be included in the list of references. This is given as follows in the text:

"Martins, according to Shepstone[6], discovered that . . . . . "

Personal (not "private") **communications and unpublished work,** as well as any unpublished proceedings or reports, are cited **in the text only and NOT in the reference list** in the following way:

". . . . . as was demonstrated recently (J Smith 1977 Veterinary Research Institute, Onderstepoort, personal communication)."

". . . . . treatment was successfully applied (P Jones 1977 Unpublished work, presented at 1977 Biennial Scientific Congress, South African Veterinary Association, Grahamstown)."

"The first case of the disease was recorded in this area in 1977 (Division of Veterinary Services, Report by State Veterinarian, Ixopo 1978, File 381/2)."

In each case a **meaningful** address and/or reference should be given so that the person or report can be traced by a reader.

22.3 List of References

The list is arranged **alphabetically** according to the authors and **chronologically** (if an author or authors have more than one publication referred to). The individual references are then **numbered** accordingly.

The most convenient way of compiling a list of references, when composing an article, is to write each complete reference in its correct and final format on a separate card. During the initial drafting of the article each card gets a temporary pencilled reference number – usually in numerical and sequential order, but any number not previously used will do – and the reference in the text receives the same number which is also noted in pencil. In this way, new references can be added or old references deleted during the drafting of the article. When writing the final copy before typing, the reference cards are placed in alphabetical order and given their new numbers. The necessary changes are easily made in the text. The typist types the references directly from the set of cards – the tedious task of writing out the list yourself is thus obviated.

The **number of references** used in the usual type of article should **not exceed 20.** If it is desired to include more than 20, permission must first be obtained from the Editor.

If possible, rather than giving 10 references for a particular fact, make use of one recent review as a single reference. *The impact of an article does not depend on the number of references used! We can assume certain facts* of life, e.g. that mastitis in cows is caused by Streptococcus agalactiae – there is then no need to write, e.g. "It is well known that a common form of mastitis in cows is caused by Streptococcus agalactiae[5 6–11 13 20]."

Personal communications, citations and unpublished observations are not references and therefore do not appear in the list of references (vide supra).

Examples of the style to be used in the reference list are as follows:

Example 1: Reference of an Article in a Journal

1. Du Toit A E, MacDonald J 1975 The excretion of Escherichia coli by pigs under stress. Journal of the South African Veterinary Association 46: 3–15

Notice here:

1. Where upper and lower case (i.e. capitals and small letters) is used. The titles of articles appear in lower case only except where spelling rules demand otherwise.

2. The punctuation – or virtual lack of it.
3. The absence of "and" or the ampersand (&) between authors.
4. The use of a first name of an authoress falls away here – only her initials are used.
5. Authors' surnames must be rendered exactly as spelt (see also 17), including particles or prefixes such as Le, De, Van, Von, O' etc. and alphabetized accordingly. Mc must not be alphabetized under Mac.
6. The name of the journal is given in full.
7. In the name of the journal all nouns and adjectives start with a capital.
8. The **number** of a volume issue will only be necessary (in brackets) in those instances in which each issue of a volume starts from page 1, as happened in Vol 39 of our Journal – 39(2): 45–47
9. First and last page numbers of each reference must be given.
10. Anonymous authors are indicated by their status, e.g. Editorial; Director of Veterinary Services; Chief Librarian; Royal Commission on .....; Joint Committee on .....; World Health Organization .....; Republic of South Africa, Department of Agricultural Technical Services .....; Republic of South Africa, Government Notice No. .....; etc. Such titles or names are arranged alphabetically with those of named authors.
11. Terms such as ibid., idem, op. cit. and loc. cit. are not to be used as substitutes for complete references.

Example 2: References of an article in a Text Book and of a Text Book

2. O'Connor P, Smith J, Jones C 1978 Symptoms of stress in pigs. In: Roos T, Marais M (ed.) Stress in Man and Animals 2nd edn Academic Press, New York: 62–92
3. Smith P, Jones C, Hunt D 1972 Veterinary Pathology 4th edn Lea & Febiger, Philadelphia

Notice here:

1. The use of upper and lower case. The chapter or article "Symptoms of stress in pigs" in the book is given in lower case only except where spelling rules dictate otherwise, but in the title of the book all nouns and adjectives start with a capital.
2. The publisher's name is given first and then the main city in which the publishing house is situated.
3. The use of the colon after "In:" and before the page numbers.

23. Plurals

Where possible the English equivalent of plurals for foreign (Latin words) should be used, unless the Latin word has already been accepted or the English is very clumsy, e.g. lumen – lumens (not lumina), alveolus – alveoli.

A photograph in an article is referred to as a figure which in the text is abbreviated, in the singular and plural, to Fig. 1; Fig. 1–20.

24. Figures and Tables

There are Figures (abbreviation, e.g. Fig. 1–6) and Tables. Use the ampersand (&) if necessary, e.g. Tables 14 & 15; Fig. 1 & 2. Photographs of e.g. an animal, a histological section or a radiograph are considered as figures, as well as graphs, histograms, maps, etc.

All figures and tables should be submitted individually on separate sheets of paper and should not be incorporated in the text. Authors must indicate where they are to be placed in the text as follows:

"These achievements are something that we as a Profession and as an Association can look back on with a measure

Insert Table 3 about here

of pride and satisfaction. We should not, however, rest on our laurels but should proceed in the future in . . ."

Obviously the printer will have to position a figure or table according to the lay-out of the page.

Figures and tables will be adjusted in size to a width of one or 2 columns of the Journal. Please bear these proportions in mind when preparing them. Original drawings, graphs, histograms, maps, etc. should not be wider than 3 column widths and should be executed in black ink on a plain white surface. All lettering (use preferably printed letters, e.g. Letraset), numbers, shaded areas and lines on them should be distinct so that even after photostating and possible reduction in size they will be clearly legible and/or visible.

The Editor will instruct the printer when a table or figure will be over one column or 2 columns.

Example of a graph:

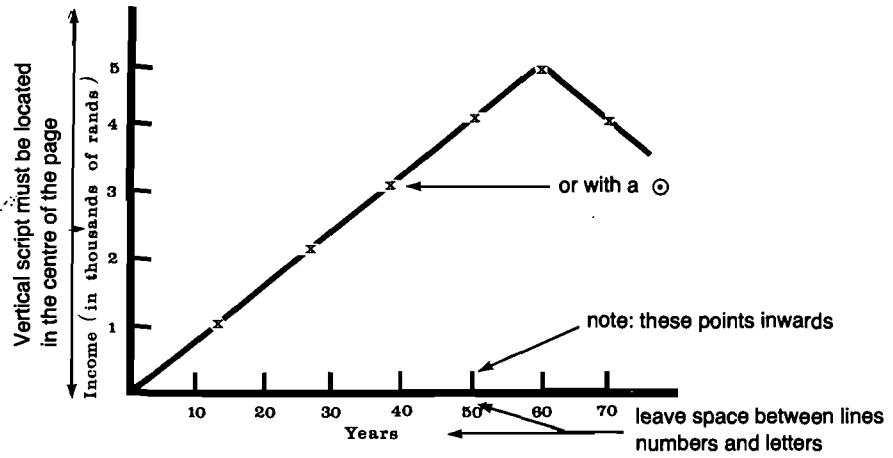

Fig. 1: Average annual income of the polulation compared to the age.

Note:

1. Graphs will be placed over 1 or 2 column widths. Use correct proportions.
2. All lettering and lines must be legible and/or visible even after possible reduction in size.
3. Execute in black ink on plain white paper.
4. Please do not annotate excessively on the graph itself.

Always write **lightly in pencil on the back** of a figure or table its number and author, e.g. "Fig. 2, Bigalke et al.", and **indicate which is the TOP** along the upper margin also on the back.

Composite plates of photographs should be prepared in the required proportion of a journal page or part thereof. Photographs for composite plates should exhibit an equal degree of a relatively high contrast for clear reproduction by the printer. Each photograph in the plate should be labelled distinctly with white or black (i.e. contrasting) printed numbers (e.g. Letraset) on the face side with the figure number: 1,2,3,4, etc.

If you wish to indicate e.g. morphological details on an electron microphotograph, use printed letters or arrows (such as Letraset) in a contrasting colour, i.e. white letters on black or dark background and vice versa. When used, such letters or arrows must be explained in the legend.

Radiographs are to be referred to as radiographs and not as X-rays.

Always protect photographs from being scratched and folded by placing in envelopes between 2 sheets of cardboard.

Photographs should preferably be in the form of glossy prints with relatively high contrast.

The number of figures and tables may be limited at the Editor's discretion unless the author contributes to the cost of reproduction. Authors (or their sponsors) are expected to contribute towards the cost of colour reproductions. Sponsors may be acknowledged by inserting their names beneath the figures or in the legends.

Legends to figures: **These should all be typed on the same sheet of paper in numerical order** (and **not** incorporated in the body of the text). In the Journal they will appear **beneath** the figure, e.g.:

Fig. 5: Photomicrograph of a superficial lesion after 8 weeks. Note the infolding edge of the lesion (arrow) and cell division occurring at the surface of the lesion. Haematoxylin and eosin stain. X60

Legends to tables: These appear **above** the table, e.g.:

Table 2: Efficacy claims in cattle for fenbendazole dosed at 7,5 mg/kg live mass

The **number and size of tables should be limited** as far as possible. If you can illustrate your point in a graph or histogram rather than in a table, please do so. They are easier to understand.

Please fill in "empty spaces" in a table with a dot or dash. This facilitates type-setting and obviates mistakes and confusion.

Each table with its legend must be typed on a separate sheet of paper.

25. Acknowledgements

Keep the acknowledgements as short as possible.
We will assume that all contributions submitted will have received the necessary permission of the authority concerned, if any, for publication to occur. This will therefore not be printed.

26. Book Reviews

Book reviews should be typed as follows:

Example 1

BOOK REVIEW BOEKRESENSIE

RESTRAINT AND HANDLING OF WILD AND DOMESTIC ANIMALS

MURRAY E. FOWLER

1st Edn. The Iowa State University Press, Ames, Iowa 50010. 1978, pp VI and 332, illustrations 744 and numerous tables, Price US $26.00 (ISBN 0–8138-1890-7).

This book describes the ..... end of the road.

J. Burger

The initials and surname of the reviewer will be given at the end of the review.

Book reviews should be brief but as informative as possible. The information concerning the edition number, publishers, price, etc., which appears immediately below the name(s) of the author(s) should be as comprehensive as possible. This enables a reader to order the book for a library or from a book store without any difficulty.

Example 2

BOOK REVIEW BOEKRESENSIE

SURVEILLANCE OF ANTIBIOTIC RESISTANT ENTEROBACTERIA
TECHNICAL REPORT SERIES No. 624
REPORT OF A WHO MEETING

World Health Organization, Geneva. 1978 pp 54. Price Sw. fr. 6 (ISBN 92 4 1206241). Available in RSA from Van Schaik's Bookstore (Pty) Ltd, P.O. Box 724, 0001 Pretoria.

The uncontrolled and excessive use ..... drugs was not effective.

J. Burger

27. Letters to the Editor

Letters to the Editor should be typed as follows:

TO THE EDITOR AAN DIE REDAKSIE

DIE STEDELIKE VEEARTS – 'N VERLEENTHEID OF GELEENTHEID?

Die afgelope tyd is heelwat ..... veeartse opgelei.

J.S.J. Odendaal
Olive Grinterrylaan 2
Fichardtpark
9301 Bloemfontein

Please note: There should be no "Dear Sir", etc. If the writer has not given the letter a brief heading or title, the Editor will. This will also appear in the list of contents on the front cover.

The address of the writer comes after his name at the end of the letter. We will not use the author's titles and/or qualifications.

28. Feature Page

The format of this page may vary to fulfil a particular purpose, but in general it should be typed and arranged as follows:

FEATURE PAGE TREFFERBLAD

Place Fig. here over two columns

SQUAMOUS CELL CARCINOMA IN A HORSE

In January 1978 a Welsh.....

Submitted by: S.W. Petrick
Department of Surgery
Faculty of Veterinary Science
University of Pretoria
P.O. Box 12580
Onderstepoort
0110

PLAVEISELKARSINOOM IN 'N PERD

Gedurende Januarie 1978 is 'n Walliese .....

Ingestuur deur: S.W. Petrick
Departement Chirurgie
Fakulteit Veeartsenykunde
Universiteit van Pretoria
Posbus 12580
Onderstepoort
0110

Please note: It is desirable, but not imperative, that such contributions should be in both English and Afrikaans. If possible, they should fit onto one journal page.

29. Case Reports, Research Notes, Short Communications and Clinical Communications

The format of these may vary to fit the particular case. A set pattern cannot be prescribed for them. Authors are allowed some latitude.

An **abstract** must, however, be supplied with each case report, etc.

30. General

30.1 Before submitting the manuscript for publication, it should be read again with the utmost critical attention and preferably also given to a colleague who has some experience of writing articles, to criticize. Not even the most inexperienced writer can be forgiven for writing mmol/l in 3 different ways in one article! Authors should avoid needless repetition and verbosity and should keep their articles as short as possible.

A little extra time spent on the manuscript may in the end save a considerable delay before publication. It will also lighten the burden of the Editorial Committee.

30.2 *Reprints* should be ordered when you have been informed of the volume and number of the Journal in which your article will appear. This is usually just before publication. The senior author receives 25 reprints free of charge.

30.3 *Copyright:* If you wish either to reproduce replicas of figures or tables or to quote extensively from another article, written permission from the publishers or other proper authority must first be obtained. This permission must accompany your article when it is submitted to the Editor for the first time. It must also be acknowledged in the text.

ADDENDUM TO GUIDE FOR AUTHORS

SI UNITS AND SYMBOLS

A little more than 2 decades ago South Africa decided to change to the metric system. Subsequently the *Système International d'Unités* (International System of Units, SI units) which is based on the metric system was introduced. This system was introduced to provide a standardized means of expressing results. SI units are being used internationally and are taught at our schools and universities. It is therefore our duty as a profession to adapt (if only slowly) to this internationally recognized system.

The following metric prefixes and their symbols are used to express the magnitude of a quantity:

Factor	Prefix	Symbol
10^{12}	tera –	T
10^{9}	giga –	G
10^{6}	mega –	M
10^{3}	kilo –	k
10^{2}	hecto –	h
10^{1}	deca –	da
10^{-1}	deci –	d
10^{-2}	centi –	c
10^{-3}	milli –	m
10^{-6}	micro –	μ
10^{-9}	nano –	n
10^{-12}	pico –	p
10^{-15}	femto –	f
10^{-18}	atto –	a

The following are some of the more frequently used SI units and their symbols:

Time

1 s, 2 s	1 second, 2 seconds
1 min, 2 min	1 minute, 2 minutes
1 h, 7 h	1 hour, 7 hours
1 d, 15 d	1 day, 15 days

No abbreviations for: week, weeks
month, months
year, years

The **time of day** will be written e.g.
08h00 for 8 a.m.
16h30 for 4.30 p.m. etc.

The **date** will be written e.g. 12 January 1980
The **day** be written e.g. Tuesday

Length

The base unit for length is a metre. = m

1 nanometre = 1 nm = $\frac{1}{1\,000\,000\,000}$ m (10^{-9})

1 micrometre = 1 μm = $\frac{1}{1\,000\,000}$ m (10^{-6})

1 millimetre = 1 mm = $\frac{1}{1\,000}$ m (10^{-3})

1 kilometre = 1 km = $\times$ 1 000 m (10^{3})

1 centimetre = 1 cm (N.B. used only in the clothing and textile industries and for measuring human height, animal length and girth).

Area

mm^2	square millimetre
cm^2	square centimetre
m^2	square metre
km^2	square kilometre
ha	hectare = 10 000 m^2

Volume

The base unit for volume of liquids or gases is a litre = l (always underline the l when it stands alone – this will then be italicized in print otherwise l may be mistaken for one).

1 femtolitre = 1 fl (10^{-15})
1 microlitre = 1 μl (10^{-6})
1 millilitre = 1 ml (10^{-3})
1 kilolitre = 1 kl (10^{3})

(Cubic metre (m^3), cubic decimetre (dm^3) and cubic centimetre (cm^3 – never cc) are used for so-called hard capacities such as the luggage compartment of a car, the piston displacement of an engine).

Mass

The base unit for mass (note **not** weight) is the

kilogram = kg = × 1 000 g (10^3)
1 milligram = 1 mg (10^{-3})
1 gram = 1 g
1 metric ton = 1 t = 1 000 kg

(Note this is a Roman t)

Mass may be determined or measured.

Speed

metres per second = m/s
kilometres per hour = km/h

Pressure

pascal = Pa
kilopascal = kPa

Temperature

The practical unit for temperature (t, the t will be italicized in print) is the degree Celsius (°C) where 0 °C is the freezing point and 100 °C is the boiling point of water at sea level. Celcius is used for measuring body, water, climatic temperature, etc.

The kelvin (K) is the SI unit for absolute temperature or dynamic temperature (T, the T is italicized). The symbol ° is not used as in the case of Celsius but is simply written as e.g. 210,5 K. 1 K is equal to 1 °C but the kelvin scale starts at absolute zero (–273 °C). Thus 0 °C equals 273,15 K and 100 °C = 373,15 K. Kelvin is used in technology and calculations involving gas laws.

Amount and concentration of a substance

The amount of a substance is described in mole (SI symbol = mol). The mole amount of a substance or a system is that amount which contains as many elementary entities as there are atoms in 0,012 kg of C^{12}.

Concentration of a substance is expressed in mole per litre typed as mol/l. Where the molecular mass of a substance is unknown (e.g. FSH and LH in some species) concentration is expressed as mass per litre which is written as g/l. Note that the unit of volume used in both cases is the litre and **not** a millilitre or decilitre, etc.

1 micromole per litre = 1 μmol/l
1 millimole per litre = 1 mmol/l
1 picogram per litre = 1 pg/l

Radioactivity and ionising radiation

The becquerel is the SI unit of radioactivity (SI symbol Bq). This unit replaces the previously used curie (Ci).

1 Ci = 37 GBq
1 Bq = 27 pCi

The old unit for absorbed dose of ionising radiation was the rad (rad). This is replaced by the SI unit gray (Gy). The gray per second (Gy/s) is absorbed dose rate.

1 rad = 0,01 Gy
1 Gy = 100 rad

The coulomb per kilogram (C/kg) is the SI unit of exposure. It replaces the roentgen (R). The exposure rate is expressed as coulomb per kilogram second (C/kg.s)

1 R = 0,258 mC/kg
1 C/kg = 3 876 R

General notes

Note that there is no difference between the singular and plural and that no full stops are used. Note also that symbols written in small letters do not change to capitals when used in headings which are written with capital letters and vise versa e.g. THE USE OF PHENOTHIAZINE AT A DOSAGE RATE OF 10 mg/kg BODY MASS and **not** 10 MG/KG or 10 Mg/Kg BODY MASS.

A comma is used as a decimal sign.

To facilitate the reading of many digits, these should be separated into groups of 3, counting from the decimal sign towards the left and right. The groups should be separated by a small space (and never by a comma or a point or other means), e.g. 1 003 004,123 456

In numbers less than unity a zero should precede the decimal sign, e.g. 0,239 not ,239.

Note the space between the numeral and the unit of measurement:

5 g	(not 5g)
7 ml	(not 7ml)
10 °C	(not 10°C)
20 kPa	(not 20kPa)

Laboratory reports and SI units

The more commonly performed laboratory tests are tabulated in Tables 1–8. Included in the tables are the currently accepted name (and where applicable, abbreviation) of the substance tested for, the SI unit and old unit more commonly used and the conversion factor from the SI unit to the old unit and vice versa. The abbreviated prefixes are explained at the bottom of each table. Note that in some cases P (plasma) is interchangable with S (serum) depending on whether the determination was carried out on plasma or serum.

Note also that amounts of substances present in urine or faeces are the amounts excreted over a 24-hour period (daily urine, dU; daily faeces, dF) rather than expressing them in the form of concentrations. The reason for this is that urine and faeces volumes and therefore concentrations vary considerably.

Enzymology

The more commonly determined serum enzymes together with their recommended names and abbreviations, biochemical names and one of the previously used names and abbreviations are given in Table 8. In addition, each enzyme listed has an EC Code Designation. This was a number given to each enzyme by the Enzyme Commission appointed by the Union of Biochemistry to standardize the nomenclature of enzymes. EC is the abbreviation for Enzyme Commission. The first number of the code indicates the class of the enzyme, the second and third the subclass and sub-sub-class, and the last number, the serial number of the enzyme in the subclass.

In addition to the vast array of names given to enzymes, more confusion was added by using different units for expressing their activity. The unit of enzyme activity has now been precisely defined as:

"The amount of enzyme which converts 1 μmol (micromole) of substrate in one minute under optimised or specified conditions". The standardized temperature for the assay of enzymes was laid down as 25 °C, although most European countries now standardize at 37 °C for the purpose of Clinical Chemistry. The unit of volume is one litre.

Thus the activity of an enzyme may be reported as follows:

"The activity of ALT was 25 U/l at 37 °C using the method of ..... " Milli- or microunits may also be used. Note that the method must always be reported. When comparing results, comparisons are only valid when the same methods were employed.

Table 1: HAEMATOLOGY

Component and abbreviation	SI unit	Old unit	Conversion factor SI to old	Conversion factor Old to SI
Haemoglobin conc B-Hb	g/l	g/dl	x 10	x 0,1
Haematocrit (PCV) B-Ht	Proportion of 1 e.g. 0,35	percentage e.g. 35%	x 100	x 0,01
B-Erythrocytes B-RBC	x 10^{12}/l	x 10^6/μl or x 10^6/mm^3	x 10^{-6}	x 10^6
B-Reticulocytes (absolute count)	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6
E-Reticulocytes (differential count)	Proportion of 1 e.g. 0,1	percentage e.g. 10%	x 100	x 0,01
Mean corpuscullar volume E-MCV	fl	μm^3	x 1	x 1
Mean corpuscular haemoglobin E-MCH	pg	pg	No change	
Mean corpuscular haemoglobin concentration E-MCHC	g/dl	percentage e.g. 35%	x 1	x 1
Erythrocyte sedimentation rate B-ESR (Westergren or Wintrobe 1 h at 20 ± 3 °C, horse 20 min)	mm	mm	No change	
B-Leukocytes B-WBC	x 10^9/l	x 10^3/μl or x 10^3/mm^3	x 10^{-6}	x 10^6
Absolute individual white cell counts				
B-Neutrophils	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6
B-Eosinophils	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6
B-Basophils	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6
B-Lymphocytes	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6
B-Monocytes	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6
Differential white cell counts	Proportion of 1 e.g. 0,41	percentage e.g. 41%	x 100	x 0,01
Lkc-Neutrophils				
Lkc-Eosinophils	proportion of 1	percentage	x 100	x 0,01
Lkc-Basophils	proportion of 1	percentage	x 100	x 0,01
Lkc-Lymphocytes	proportion of 1	percentage	x 100	x 0,01
Lkc-Monocytes	proportion of 1	percentage	x 100	x 0,01
B-platelets	x 10^9/l	x 10^3/μl	x 10^{-6}	x 10^6

B = blood, PCV = packed cell volume, E = erythrocyte, Lkc = leukocyte.

Table 2: HAEMOSTASIS

Component and abbreviation	S I unit	Old unit	Conversion factor S I to old	Old to S I
Pt-Capillary bleeding time	min	min	No change	
P-Fibrinogen	g/l	g/dl	x 0,1	x 10
S-Fibrinogen degradation products P-FDP	mg/l	mg/dl	x 0,1	x 10
P-Prothrombin time	s	s	No change	
P-Kaolin cephalin clotting time	s	s	No change	

Pt = patient, P = plasma, S = serum

Table 3: DETERMINATIONS ON PLASMA OR SERUM

Component and abbreviation	SI unit	Old unit	Conversion factor SI to old	Old to SI
(a) Plasma or serum proteins				
Total serum proteins S-TSP or Total plasma proteins P-TPP	g/l	g/dl	x 0,1	x 10
S-Albumin	g/l	g/dl	x 0,1	x 10
S-Globulins	g/l	g/dl	x 0,1	x 10
S-α-Globulins	g/l	g/dl	x 0,1	x 10
S-β-Globulins	g/l	g/dl	x 0,1	x 10
S-γ-Globulins	g/l	g/dl	x 0,1	x 10
Albumin globulin ratio A/G	eg. 0,9:1		No change	
S-Caeruloplasmin	g/l	g/dl	x 0,1	x 10
S-Lipoproteins	g/l	mg/dl	x 100	x 0,01
S-Transferrin	g/l	g/dl	x 0,1	x 10
(b) Other plasma or serum organic constituents				
S-Bilirubin (total) and S-Bilirubin (conjugated)	μmol/l	mg/dl	x 0,058	x 17,1
S-β-Carotene	μmol/l	g/dl	x 55,6	x 0,0186
S-Cholesterol (total)	mmol/l	mg/dl	x 38,7	x 0,0259
S-Creatinine	μmol/l	mg/dl	x 0,011	x 88,4
P-Glucose (fPt)	mmol/l	mg/dl	x 18	x 0,056
S-Ketones (total, as acetone)	μmol/l	mg/dl	x 0,058	x 17,2
S-Lactate	μmol/l	μg/dl	x 8,93	x 0,112
S-Lipids (total)	g/l	mg/dl	x 100	x 0,01
P-Osmolality	mmol/l	mOsm/kg	x 1	x 1
S-Pyruvate	μmol/l	ng/dl	x 0,0087	x 115
S-Triglycerides (total)	mmol/l	mg/dl	x 87,5	x 0,0113
S-Urate (uric acid)	mmol/l	mg/dl	x 17	x 0,0598
S-Urea	mmol/l	mg/dl	x 6	x 0,167
		BUN:	to x 2,8	from x 0,251
S-Vitamine A	μmol/l	μg/dl	x 29	x 0,035
S-Vitamine B_{12}	pmol/l	pg/dl	x 1,35	x 0,738
BSP retention	%	%	No change	
BSP T½	min	min	No change	
(c) Hormones in plasma or serum				
P-Adrenaline	nmol/l	μg/dl	x 0,183	x 5,46
P-Aldosterone	nmol/l	μg/dl	x 0,361	x 2,77
P-Corticotropin P-ACTH	ng/l	ng/l	No change	
P-Cortisol	nmol/l	μg/dl	x 0,036	x 27,6
P-Dopamine	nmol/l	μg/dl	x 0,153	x 6,54
P-Follitropin P-FSH	μg/l	ng/ml	x 1	x 1
P-Lutropin P-LH	μg/l	ng/ml	x 1	x 1
P-Noradrenaline	nmol/l	μg/l	x 0,169	x 5,91
P-Oestradiol	nmol/l	μg/l	x 0,272	x 3,67
P-Oestriol	nmol/l	μg/l	x 0,288	x 3,47
P-Oestrone	nmol/l	μg/l	x 0,270	x 3,70
P-Oestrogens (total)	nmol/l	μg/l	x 0,290	x 3,46
P-Progesterone	nmol/l	ng/dl	x 31,4	x 0,0318
S-Testosterone	nmol/l	ng/dl	x 29	x 0,0346

Table 3 is continued on next page

Table 3 continued: DETERMINATIONS ON PLASMA OR SERUM

Component and abbreviation	SI unit	Old unit	Conversion factor SI to old	Conversion factor Old to SI
S-Thyroxine S-T_4	nmol/l	µg/dl	x 0,08	x 12,85
S-Triiodothyronine S-T_3	nmol/l	µg/l	x 0,65	x 1,535
S-Free thyroxine index S-FTI	nmol/l	µg/dl	x 0,08	x 12,85
S-%T_3-^{125}I retention	percentage	percentage	No change	
(d) Inorganic constituents of plasma or serum				
P-Bicarbonate	mmol/l	mEq/l	x 1	x 1
P-Calcium (total)	mmol/l	mg/dl	x 4	x 0,25
P-Chloride	mmol/l	mEq/l	x 1	x 1
P-Copper	µmol/l	µg/dl	x 6,3	x 0,157
S-Iron	µmol/l	µg/dl	x 5,5	x 0,179
B-Lead	µmol/l	µg/dl	x 20	x 0,0483
P-Magnesium	mmol/l	mg/dl	x 2,4	x 0,411
P-Phosphate (inorganic)	mmol/l	mg/dl	x 3,1	x 0,323
P-Potassium	mmol/l	mEq/l	x 1	x 1
P-Sodium	mmol/l	mEq/l	x 1	x 1

P = plasma, S = serum, fPt = fasting patient

Table 4: BLOOD GASES AND BICARBONATE

Component and abbreviation	SI unit	Old unit	Conversion factor SI to old	Conversion factor Old to SI
aB-Carbon dioxide aB-Pco_2 (vB)	kPa	mmHg	x 7,5	x 0,133
aB-Oxygen aB-Po_2 (vB)	kPa	mmHg	x 7,5	x 0,133
aB-Base excess	mmol/l	mEq/l	x 1	x 1
aB-Standard bicarbonate	mmol/l	mEq/l	x 1	x 1
aB-Actual bicarbonate	mmol/l	mEq/l	x 1	x 1
aB-Buffer base	mmol/l	mEq/l	x 1	x 1

aB = arterial blood, vB = venous blood

Table 5: URINE CONSTITUENTS

Component and abbreviation	SI unit	Old unit	Conversion factor SI to old	Conversion factor Old to SI
dU-Aldosterone	nmol	µg	x 0,361	x 2,77
dU-Arsenic	µmol	µg	x 74,6	x 0,0134
dU-Calcium	mmol	mg	x 40	x 0,025
dU-Catecholamines (as noradrenaline)	µmol	µg	x 169,2	x 0,00591
dU-Copper	µmol	µg	x 63	x 0,0157
dU-Cortisol	nmol	µg	x 0,36	x 2,76
dU-Creatinine	mmol	g	x 0,113	x 8,84
dU-Glucose	mmol	mg	x 180	x 0,0056
dU-Lead	µmol	µg	x 207	x 0,00483
dU-Oestradiol	nmol	µg	x 0,272	x 3,67
dU-Oestriol	nmol	µg	x 0,288	x 3,47
dU-Phosphate (Inorganic)	mmol	g	x 0,031	x 32,3
dU-17-Total-oxogenic steroids (as DHEA)	µmol	mg	x 0,288	x 3,47
dU-17-Oxosteroids (as DHEA)	µmol	mg	x 0,288	x 3,47
dU-Pregnanediol	µmol	mg	x 0,321	x 3,12
dU-Urate	mmol	g	x 0,17	x 5,98
dU-Urea	mmol	g	x 0,0598	x 16,7

dU = daily urine (24-hour period)

Table 6: FAECES CONSTITUENTS

Component	SI unit	Old unit	Conversion factor SI to old	Conversion factor Old to SI
dF-Calcium	mmol	mg	x 40	x 0,025
dF-Fat (as fatty acid)	mmol	g	x 0,284	x 3,52
dF-Nitrogen	mol	g	x 14	x 0,0714
dF-Phosphate	mol	g	x 31	x 0,0323
dF-Urobilinogen	mmol	mg	x 595	x 0,00168

dF = daily faeces (24-hour period)

Table 7: CEREBROSPINAL FLUID CONSTITUENTS

Component	SI unit	Old unit	Conversion factor SI to old	Conversion factor Old to SI
Sf-Chloride	mmol/l	mEq/l	x 1	x 1
Sf-Glucose	mmol/l	mg/dl	x 18	x 0,056
Sf-Protein	g/l	mg/dl	x 100	x 0,01

Sf = spinal fluid

Table 8: ENZYMOLOGY

E C Code Designation	Recommended name	abbreviation	Biochemical or systemic name	Previous name	abbreviation
3.1.3.2	Phosphatase, acid	ACP	Orthophosphoric monoester phosphohydrolase	Acid phosphatase	SAcP
3.1.3.1	Phosphatase, alkaline	ALP	Orthophosphoric monoester phosphohydrolase	Alkaline phosphatase	SAP
4.1.2.13	Aldolase	ALS	D-Fructose-1,6,-biphosphate glyceraldehyde 3-phosphate-lyase	Aldolase	AlD
2.6.1.2	Alanine transaminase	ALT	L-Alanine:2-oxoglutarate aminotransferase	Glutamic pyruvic transaminase	SGPT
2.6.1.1.	Aspartate transaminase	AST	L-Aspartate:2-oxogluterate aminotransferase	Glutamic oxalacetic transaminase	SGOT
3.1.1.7/8	Cholinesterase	CHS	Acetylcholine acyl-hydrolase	Cholinesterase	CHE
2.7.3.2	Creatine kinase	CK	Creatine phosphotransferase	Creatine phosphokinase	CPK
1.4.1.3	Glutamate dehydrogenase	GMD	L-Glutamate: NAD oxidoreductase	Glutamate dehydrogenase	GLDH
3.2.1.1	Amylase	AMS	α-1,4-Glucan glucanohydrolase	α-Amylase	
1.1.1.42	Isocitrate dehydrogenase	ICD	L-Isocitrate: NADP oxidoreductase	Isocitrate dehydrogenase	ICDH
2.3.2.2	γ-Glutamyltransferase	GGT	L-γ-Glutamyltransferase	Gamma-glutamyl transpeptidase	GGTP
1.1.1.27	α-Hydroxybutyrate dehydrogenase	HBD	L-Lactate: NAD oxido-reductase-l-isoenzyme	Hydroxybutyrate dehydrogenase	HBDH
1.1.1.27	Lactate dehydrogenase	LD	L-Lactate: NAD oxidoreductase	Lactate dehydrogenase	LDH
3.1.1.3	Lipase	LPS	Triacylglycerol acyl-hydrolase	Lipase	
3.4.11.1	Leucine arylamidase		α Aminoacyl-peptide hydrolase	Leucine amino-peptidase	LAP
1.1.1.37	Malate dehydrogenase	MD	L-Malate: NAD oxidoreductase	Malate dehydrogenase	MDH
2.1.3.3	Ornithine carbamoyltransferase	OCT	L-Ornithine carba-moyltransferase	Orinithine carbamoyl transferase	OCT
1.1.1.14	Sorbitol dehydrogenase	SD	L-Iditol: NAD oxidoreductase	Sorbitol dehydrogenase	SDH
2.7.1.40	Pyruvate kinase	PK	Pyruvate 2-O-phosphotransferase	Pyruvate kinase	PK
3.4.21.4	Trypsin	TPS	Peptide-peptidohydrolase	Trypsin	

EC = Enzyme Commission

ACKNOWLEDGEMENTS

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A REPORT ON THE OCCURRENCE OF SEPTICAEMIA CAUSED BY *PASTEURELLA MULTOCIDA* TYPE E IN CATTLE FROM SOUTHERN AFRICA

STELLA S. BASTIANELLO* and M.R. JONKER**

ABSTRACT: Bastianello S.S.; Jonker M.R. **A report on the occurrence of septicaemia caused by *Pasteurella multocida* type E in cattle from Southern Africa.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 99–104 (En) Veterinary Research Institute, 0110 Onderstepoort, South Africa.

Haemorrhagic septicaemia caused by *Pasteurella multocida* type E was diagnosed at post-mortem examination in a bovine originating from South West Africa. This is the first report of this disease occurring in South West Africa. The history, clinical symptoms and gross and microscopic pathology of this case are described. The pathologic features included generalized congestion, subcutaneous oedema especially of the submandibular area, fibrinous arthritis, tendovaginitis and myositis with an accompanying lymphadenitis of the regional lymph nodes and a haemorrhagic pleuritis and pericarditis. The epizootiology of the disease in South West Africa as compared with that in Central Africa and Asia, where the disease is common, is discussed.

A case of septicaemia caused by *P. multocida* type E in a calf from a group of calves originating from the Transvaal, is also reported. A post-mortem examination on this calf revealed moderate pulmonary oedema and generalized congestion of the organs and musculature. The latter lesions suggest a septicaemic condition. Other calves in this group revealed one or more of the following lesions: myositis, fibrinopurulent pneumonia or fibrinous peritonitis.

INTRODUCTION

Haemorrhagic septicaemia is the term used to denote a septicaemic and haemorrhagic disease in cattle caused by *Pasteurella multocida* of either Carter type B or Carter type E[6]. This disease occurs commonly in several Asian and Central African countries. In Asia, both cattle and buffalo can be affected and it has been recorded in Sri Lanka, Indonesia, India, Iran and the Phillipines. In these countries the disease is caused by *P. multocida* type B[6 11–14]. *P. multocida* type B was also involved in an outbreak of haemorrhagic septicaemia in calves in the United States of America. The disease is, however, not common in the USA[9].

In 1961, Perreau, when studying the disease in upper Chad in Africa, was able to show that the African form of haemorrhagic septicaemia is not caused by type B but by another type which at that stage had not yet been classified[15]. In 1963, Carter, using the indirect haemagglutination (HA) test for typing *P. multocida* strains showed that a new type which he designated type E was the cause of haemorrhagic septicaemia in cattle originating from Zaire and the Cameroon[3 16].

Since 1961, haemorrhagic septicaemia as caused by *P. multocida* type E has been reported from several countries in Central Africa and recently it was also reported to occur in Zambia[8]. Towards the end of 1978, a case of haemorrhagic septicaemia was diagnosed in a bull originating from the Gobabis area of South West Africa. *P. multocida* type E had been isolated on a former occasion from a bull from the Caprivi strip of South West Africa (Cameron C.M., Veterinary Research Institute, Onderstepoort, personal communication). Since 1978, *P. multocida* type E was again isolated in Southern Africa, this time from a calf originating from the Lichtenburg district in South Africa.

The purpose of this report is to record the clinical and, in particular, the pathological lesions caused by *P. multocida* type E as observed in cattle from Southern Africa. These are the first reported cases of *P. multocida* type E septicaemia in cattle from Southern Africa.

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HISTORY AND CLINICAL SYMPTOMS

In December 1978, a Brahman bull about 18 months of age was submitted for a post-mortem examination. The bull was one of a batch of 55 cattle (Batch 1) that originated from the Gobabis area in South West Africa. The cattle were purchased by the owners of a feedlot system situated in the Benoni district in South Africa. About 50 % of these cattle were Brahmans whilst the remainder were crossbred Simmentaler cattle. The cattle were transported by rail, which entails a journey of about 7 days. At Keetmanshoop, which lies at about a third of the way from Gobabis to Benoni, the cattle were offloaded to be fed and watered. Here, 3 of the 55 cattle, all Brahmans, were found dead, but the cause of death was not determined. When the cattle arrived at their destination near Benoni, another 3 cattle, also Brahmans, were seen to be sick.

The 3 animals that appeared sick in this batch of cattle were examined clinically. They all had high temperatures of 40 to 41 °C and showed mild inco-ordination. An extensive swelling could be seen in the submandibular area in all 3 animals. These swellings were firm on palpation and did not pit when pressure was applied. In one of these cattle, the swelling extended beyond the submandibular region down the lateral and ventral surfaces of the neck to the level of the chest.

The 3 affected cattle were treated with a tetracycline antibiotic and cortisone. There was no response to this treatment. They died 24–36 h after having been examined.

A second batch (Batch 2) of 44 cattle originating from the same area in South West Africa were dispatched a few days later to the same feedlot. This batch was also composed of about 50 % Brahman cattle and 50 % crossbred Simmentaler cattle. All the 44 cattle were treated, before being transported, with intramuscular injections of a tranquilliser containing propionylpromazine (Combelen V, Bayer) and a long-acting penicillin antibiotic, (Compropen V, Glaxo.) No animals became sick or died in this batch.

In April 1980, a crossbred Brahman calf 5 months of age was submitted for post-mortem examination. The calf was showing a severe dyspnoea and died suddenly, shortly after developing the dyspnoea. This calf was

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one of a group of calves varying in age from 5 to 12 months. They originated from a farm in the Lichtenburg district in the Transvaal. All the calves in this group were showing signs either of respiratory distress or pneumonia. A high proportion of these calves died.

MATERIALS AND METHODS

Gross Pathology

Autopsies were performed on 2 bulls from South West Africa, designated Case I and Case II and a further autopsy on the calf from the Lichtenburg district, designated Case III.

Other calves in this group were autopsied by a private practitioner.

Histopathology

Specimens of various formalin-fixed tissues and organs from Cases I, II and III were processed routinely and stained with haematoxylin and eosin (HE). In addition, Gram[10] and Giemsa staining methods were applied to selected sections of the subcutis, muscle and lung from Case II.

Bacteriological Examination

Various specimens including blood, subcutaneous tissue, lung, spleen, liver, kidney and brain were submitted for routine bacterial isolation from Cases II and III.

Smears of the subcutaneous and intermuscular fluid from Case II were examined for the presence of *Clostridium novyi* or *Clostridium septicum*[2].

Clinical Pathology

A sample of thoracic fluid from Case II was analyzed for its protein content. In addition a blood smear from the same animal was stained with a 10 % Giemsa solution for 30 min.

Mycoplasmal Isolation

Specimens of striated muscle, heart, thoracic fluid and nasal mucosa from Case II were submitted for isolation of pathogenic mycoplasmas.

Virological examination

Samples of muscle, blood and synovial fluid from Case II were submitted for routine viral isolation, in particular for bovine ephemeral fever virus.

RESULTS

Gross Pathology

A firm subcutaneous swelling extending from the submandibular area to the brisket was noted in Case I. In Case II, a similar swelling was present, but it extended only to the level of the throat. When these lesions were sectioned, the cut surface appeared as yellow, oedematous to gelatinous, inflamed tissue criss-crossed by white strands of fibrinous exudate or fibrous tissue. In both cases there was also severe oedema of the entire subcutis but especially that of the limbs and ventral areas of the chest and abdomen. In Case II, oedema was also seen in the pleura, the pulmonary interlobular septa, the perirenal fat and around the attachments of the gall bladder to the liver.

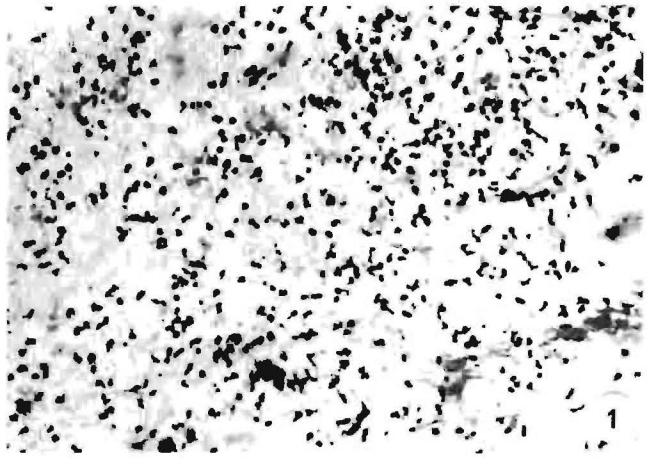

Fig. 1. Fibrinopurulent inflammatory reaction in subcutis.

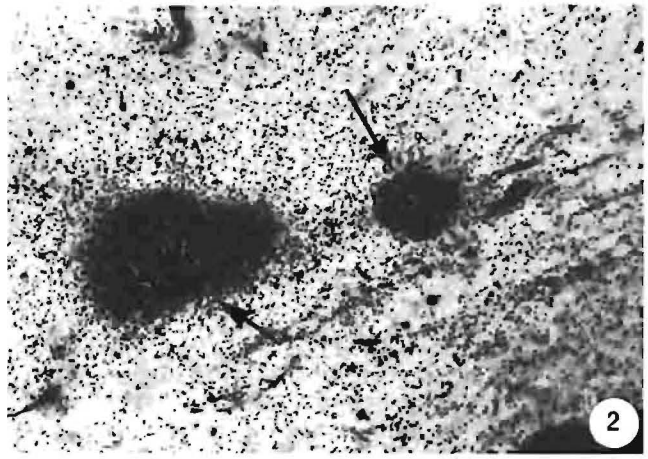

Fig. 2. Micro-abscesses within inflammatory exudate (arrows).

The underlying description concerns Case II:

There was generalized congestion of the carcass and of all the organs. The larger joints of the limbs displayed a fibrinous arthritis. The inflammation extended from the joints to involve the tendon sheaths, the intermuscular connective tissue and the muscles in the vicinity of these joints. Haemorrhages and necrosis of these muscles were seen which were associated with the fibrinous myositis. The lymph nodes draining these joints as well as the mandibular and pharyngeal lymph nodes draining the focus of cellulitis in the submandibular region revealed an acute lymphadenitis.

On opening the thoracic cavity, a severe fibrinous to haemorrhagic pleuritis as well as a moderate haemothorax were noted. The lungs were oedematous and hyperaemic. Several large haemorrhages were present subpleurally. The interlobular septa appeared prominent, dilated and milky-white in colour. The pericardium was hyperaemic and exhibited a roughened surface, indicating an acute pericarditis.

A ruminal stasis and watery intestinal contents were also noted. Besides congestion, the other organs did not show any significant lesions.

In Case III, signs attributable to a septicaemia or toxaemia were noted, i.e. a severe generalized congestion of all the organs and musculature. A moderate pulmonary oedema was also seen.

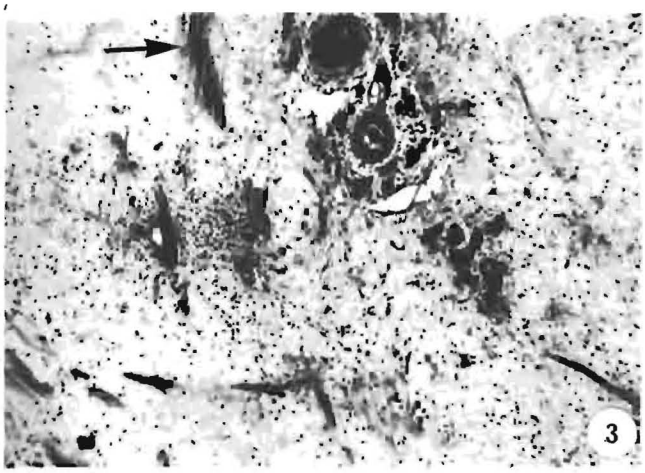

Fig. 3. Note bundles of collagen fibres around vessels and within inflammatory exudate (arrow).

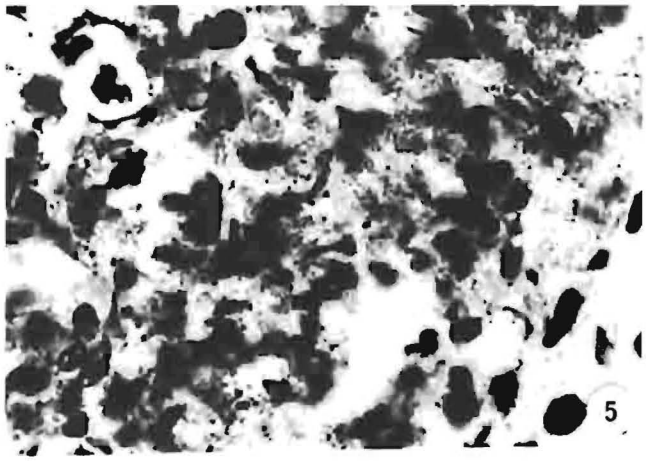

Fig. 5. Groups of bacteria visible amongst inflammatory cells. Morphology indescernible.

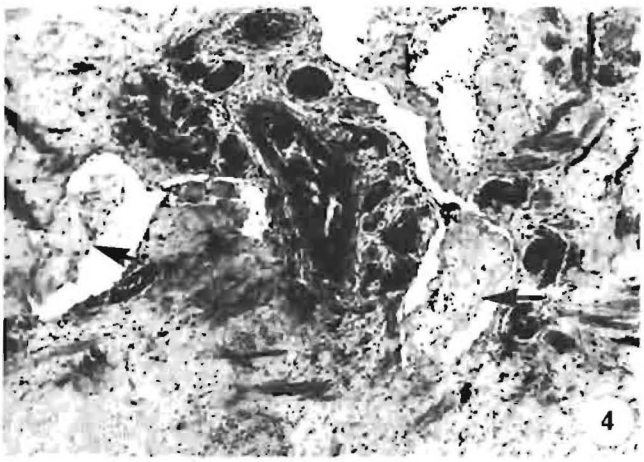

Fig. 4. Note fibrin thrombi within lymphatics (arrows).

Fig. 6. Tracts of fibrinopurulent exudate dissecting between bundles of degenerated and necrotic skeletal muscle fibres.

Several calves out of the group of calves from the Lichtenburg district were autopsied by a private practitioner. Some showed only a generalized congestion, whilst others revealed one or more of the following lesions: a fibrinopurulent pneumonia, fibrinous peritonitis or fibrinous myositis.

Clinical Pathology

Examination of the blood smear from Case II revealed the presence of several small extracellular bipolar bacterial rods.

The protein content of the thoracic fluid was 4,9 %.

Histopathology

Case I: Apart from congestion of various organs, no significant lesions were observed.

Case II: The subcutaneous and intermuscular connective tissue, the muscles in the vicinity of the larger joints of the limbs and the lungs were the tissues which revealed the most significant lesions.

The subcutaneous tissue appeared hyperaemic. Wide tracts of a serofibrinous to fibrinopurulent exudate dissected the adipose tissue. These tracts were composed of fibrin and oedematous fluid. A moderate number of red blood cells and numerous inflammatory cells were also seen in these tracts. The latter cells were mostly neutrophils but several macrophages and some lymphocytes were also present (Fig. 1). In places the neutrophils were aggregated to form microabscesses (Fig. 2). The inflammatory reaction tended to be more chronic around blood vessels as evidenced by fibroplasia and the presence of bands of collagen fibrils in the vicinity of the blood vessels (Fig. 3). Several of the larger blood vessels exhibited a purulent vasculitis seen as necrosis of the vessel wall and the infiltration of neutrophils into this necrotic material. Fibrin-type thrombi were present within several blood vessels and lymphatics (Fig. 4). Bacteria were dispersed throughout the inflammatory tissue (Fig. 5). It was difficult to discern the morphology of these bacteria in the HE-stained sections. In the sections stained with Giemsa, the bacteria were more distinct and appeared as small rods, many of which stained in a bipolar fashion. With the Gram stain the bacteria were noted mostly as Gram negative rods.

The muscles revealed a serofibrinous to fibrinopurulent fasciitis and myositis (Fig. 6). The inflammation seemed to involve primarily the intermuscular connective tissue and only secondarily the muscular tissue. The inflammatory reaction was the same as that described for the subcutis. The muscle fibres appeared as swollen, eosinophilic and degenerated or necrotic fibres or fragments thereof. The striations within the affected fibres or fragments were usually no longer evident. In places, sections of the muscle fibre had undergone lysis and appeared as empty spaces (Fig. 7), some of which, however, contained neutrophils.

The lungs were hyperaemic (Fig. 8) and small haemorrhages were present, dispersed throughout the pulmonary tissue. Plaques of fibrinopurulent exudate

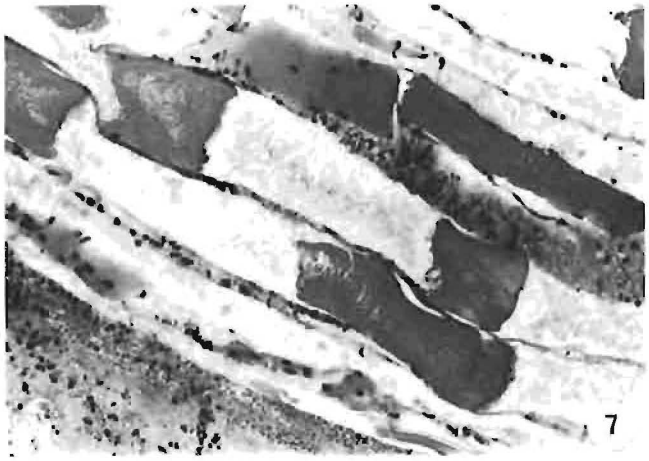

Fig. 7. Lysis of fragments of skeletal muscle fibres, noted as empty "spaces" lined only by the sarcolemma.

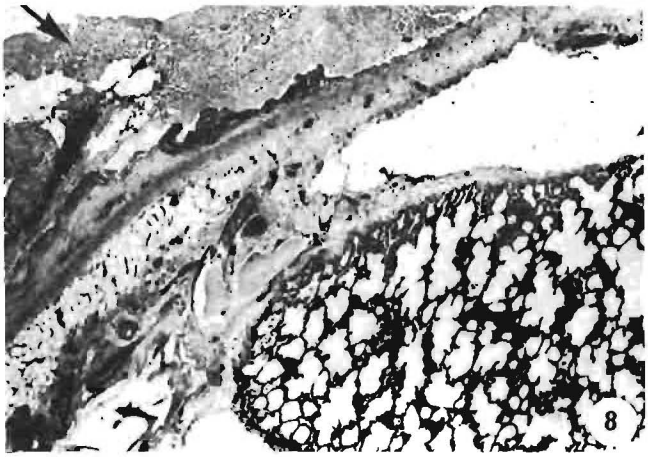

Fig. 8. A plaque of fibrinopurulent exudate on pleural surface (arrow). Note the pulmonary congestion.

could be seen to adhere to the surface of the pleura (Fig. 8). A serofibrinous to fibrinopurulent inflammatory fluid was present below the pleura and within the septa and the lymphatics. The blood vessels and lymphatics in the latter areas were dilated (Fig. 9).

The white pulp of the spleen appeared relatively acellular due to the depletion of lymphoid cells. Some of the remaining lymphoid cells as well as the dendritic or interdigitating cells of the white pulp showed karyorrhexis and necrosis. There was also lymphoid depletion in the red pulp as well as karyorrhexis of the remaining cells in the red pulp.

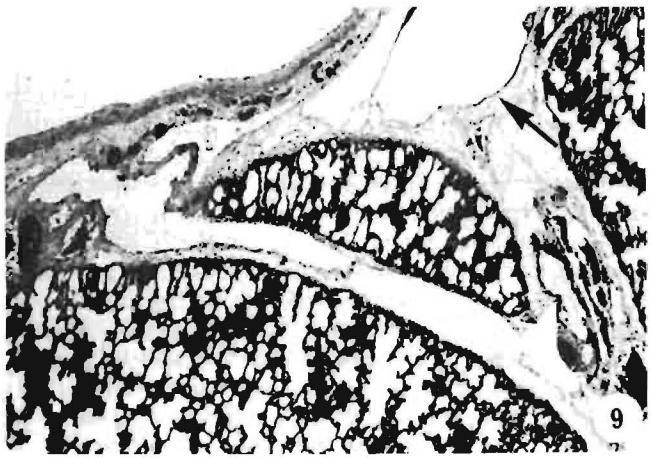

Fig. 9. Dilated lymphatics and blood vessels below pleura and within septa of lungs (arrow).

The liver, kidney and brain showed congestion and leukostasis. Degenerative changes including cloudy swelling and hydropic degeneration were present in the parenchymatous cells of the liver and kidney.

Case III: Sections of lung, liver, kidney, heart, intestine, spleen and brain revealed a severe congestion. Leukostasis within the lumens of the cerebral vessels was noted. The lungs, besides being severely congested, also revealed a moderate oedema. The spleen showed the same lesions as observed in Case II.

Bacteriology

Case II: Large numbers of *P. multocida* type E were isolated from all the tissues submitted. The specific bacterial strain isolated, Strain 4630, was confirmed to belong to type E (P. Perreau, 1979, Institut d'Elevage et de Médicine des Pays Tropicaux, France, personal communication).

No fluorescence was noted in the smears examined for the presence of *Cl. novyi* or *Cl. septicum.*

Case III: P. multocida type E was isolated in pure culture from all the specimens submitted.

Mycoplasmal Isolation

No mycoplasma were isolated from any of the tissues submitted from Case II.

Virology

No viruses could be isolated from any of the tissues from Case II.

DISCUSSION

Haemorrhagic septicaemia of cattle and buffaloes is predominantly a disease which occurs in the tropics, especially in the equatorial region of South East Asia and Central Africa[1]. In Africa the disease has been recorded in Central African countries such as Nigeria, Sudan, Chad, Cameroon, Guinea, the Ivory Coast and the Central African Republic[1 11 15 16]. In these latter countries the disease can be either epizootic or enzootic in nature. The disease in both South East Asia and Central Africa is seasonal in incidence and outbreaks usually occur during the rainy season. According to Gajapathi & D'Souza[13], outbreaks of haemorrhagic septicaemia in the Madras State of India occur following periods of high temperatures and high rainfall. The amount of rain which falls in the Gobabis and Caprivi areas of South West Africa is lower than in most of the Asian and African countries where haemorrhagic septicaemia is a problem[13 15]. However, in upper Chad both the summer temperatures and the levels of summer rainfall are similar to those occurring in the Gobabis and Caprivi regions of South West Africa. Moreover, both the latter districts lie within similar latitudes to upper Chad except that the former are in the southern hemisphere and the latter in the northern hemisphere. As the geographic situation and climatic conditions in upper Chad are similar to those in upper South West Africa, it seems likely that the situation as regards haemorrhagic septicaemia in these two countries is similar. In upper Chad, clinical cases of the disease occur only sporadically[16]. In upper South West Africa, the disease has only recently been reported to occur. It is thus reasonable to suppose that the disease in South

West Africa is also only sporadic in occurrence and this may be the reason why it has not been recorded here previously.

The cases of haemorrhagic septicaemia recorded here occurred in cattle 14–18 months of age. According to Mustafa et al.[14], the carrier rate is higher in younger cattle. On the other hand, older cattle develop an immunity following natural or artificial infection by *P. multocida* types B or E[14 16]. De Alwis et al.[6] pointed out that the disease occurred mainly in cattle between the ages of 6 and 18 months. They stated that calves below 6 months and cattle above 4 years were seldom affected[6]. The age of the Brahman bull from South West Africa (Case II) in which haemorrhagic septicaemia was diagnosed falls within these age limits.

P. multocida type E was also found to be the cause of death in a calf which was only 5 months old (Case III), but the typical lesions ascribable to haemorrhagic septicaemia were not present in this calf. It is possible that this calf was affected by a massive infection and so died peracutely before the usual lesions of haemorrhagic septicaemia had time to develop. The histological appearance of the spleen, i.e. depletion of lymphoid cells and foci of karyorrhexis, support the view of an overwhelming and massive infection. On the other hand, other calves from the same farm that died showed one or more of the pathological lesions attributable to haemorrhagic septicaemia such as a serofibrinous cellulitis, myositis or peritonitis. These calves varied in age from 5 to 12 months. Unfortunately, however, no bacterial examinations were carried out on these latter calves. Kradel et al.[12], reported that *P. multocida* type B can cause a septicaemic disease in calves. At post-mortem examination, these same authors reported that the calves may reveal a fibrinous peritonitis besides the lesions attributable to a septicaemia. On histological examination, these calves showed hyperaemia, splenic necrosis, a mild pneumonitis and a marked myositis at the experimental site of injection of *P. multocida* type B. Some of the latter histopathological lesions were seen in the calf reported in this paper. The pathological lesions seen in cases of haemorrhagic septicaemia due to *P. multocida* type B are identical to those caused by *P. multocida* type E. Hence, it seems logical to suppose that *P. multocida* type E can cause pathological lesions in calves less than 6 months of age which are similar to those caused by *P. multocida* type B as described by Kradel *et al*[12].

The occurrence of clinical cases of haemorrhagic septicaemia following predisposing or concurrent stressful conditions such as high temperatures and rainfall, worm infestations, nutritional disturbances, debilitating conditions, viral infections or bacterial infections has been recorded by several authors[1 9 12 15 16]. The Brahman bull from South West Africa (Case II) in which haemorrhagic septicaemia was diagnosed was transported by rail over a long distance. This journey may have acted as a stressful factor. Furthermore, the bull was in fairly poor condition which suggests a previous debilitating condition. The latter may have served as a further stress factor. The histological appearance of the spleen in this animal did indeed suggest that the bull was no longer able to respond to the latest immunological onslaught. In the second batch of cattle that were transported out of South West Africa, no deaths were recorded. These cattle, prior to being loaded on the train, were treated with antibiotics and a tranquilizer. The tranquilizer probably served to calm the animals and so reduce the stress caused by transport.

Of the animals transported from Gobabis (Batch 1), the 3 that became sick were all of the Brahman breed; none of the Simmentaler breed were affected. The Brahman breed is known to be highly excitable compared to European breeds. This may account for the occurrence of haemorrhagic septicaemia affecting only this breed in the cases described here. No reference as to which breed of cattle are more susceptible to haemorrhagic septicaemia could be found in the literature.

Both the adult cattle from South West Africa (Case I and II) that were autopsied showed subcutaneous oedema involving especially the submandibular region. In buffaloes, the disease is characterized by subcutaneous oedema of the submandibular region[11]. According to Anosa & Isoun[1], the subcutaneous oedema is seen in animals that survive for a few days.

A marked myositis was noted at the injection site of experimental inoculation of *P. multocida* type B[12 17]. Severe myositis was present in the larger muscles of the limbs of Case II. Trauma occurring when loading and offloading the animals and during the train journey may have created a situation similar to the trauma caused by the injection of the bacteria, and so explain the myositis noted in this bull.

According to Schels & Carter[8], haemorrhagic septicaemia can occur as a complication of contagious bovine pleuropneumonia. This should be kept in mind as the latter is a notifiable disease which can occur in similar areas in South West Africa as those in which these cases of haemorrhagic septicaemia occurred.

The incidence of *P. multocida* type E as the aetiological agent of haemorrhagic septicaemia in young adult cattle in South West Africa or septicaemia in calves in South Africa appears at present to be sporadic. It is possible that haemorrhagic septicaemia occurs more commonly in South West Africa but that it has not been recognized as such. Similarly, the incidence of septicaemia and pneumonia in calves due to *P. multocida* type E may be higher than suspected as specimens from only a small proportion of calves that die suddenly or develop a pneumonia are submitted for a bacteriological examination.

ACKNOWLEDGEMENTS

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BOOK REVIEW — BOEKRESENSIE

INFLUENCES ON ANIMAL GROWTH AND DEVELOPMENT. STUDIES IN BIOLOGY NO. 116

ROY A.L. BATT

Edward Arnold (Publishers) Ltd 1980 pp 60. Illustrations 14. Diagrams 6. Growth curves 11. References for further reading 28. Price £2-40 in the U.K.

The book deals with factors influencing the growth and development of animals. There is a chapter defining growth and development. The influence of feed and water including quantity and quality of feed, iodine, vitamins, type of feed required by herbivores, omnivores and carnivores and the effect of poisons and parasites are dealt with. Environmental influences such as temperature, micro-organisms, antibiotics, parasitism, light, stress, atmospheric pressure, gravity, seasonal differences, ionizing rays, polluted air and animal activity are discussed. Internal influences comprising growth genes, growth regulators, sex chromosomes, endocrine glands, the effect of the hormones somatotrophin and somatomedin and the interaction with other hormones are described. The final chapter deals with promoting growth and development. Illustrations, diagrams and growth curves supplement the text. There is an index of the contents and a list of references for further reading. The book is printed on good-quality paper, the print is clear and spaces between the lines make it easy to read. This study can be recommended to anyone involved in or interested in the production of food as it summarizes the different aspects of growth of animals clearly and concisely.

G.D. Sutton

CANINE PARVOVIRUS MYOCARDITIS: CLINICAL SIGNS AND PATHOLOGICAL LESIONS ENCOUNTERED IN NATURAL CASES*

STELLA S. BASTIANELLO*

ABSTRACT: Bastianello Stella S.; **Canine parvovirus myocarditis: Clinical signs and pathological lesions encountered in natural cases.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 105–108 (En) Department of Pathology, Veterinary Research Institute, 0110 Onderstepoort, Republic of South Africa.

Canine parvovirus (CPV) myocarditis was diagnosed in 11 puppies during 1979. The diagnosis was made at histopathological examination on the basis of 2 characteristic lesions: a subacute to chronic fibrous myocarditis and the presence of large, basophilic intranuclear inclusions in the cardiac myofibres.

The puppies varied from 3½ to 8 weeks of age and all died suddenly without prior symptoms except for 1 pup which developed a severe respiratory dyspnoea 12 h before death. The presence of white bands or streaks of fibrosis on the endocardial or epicardial surfaces of the ventricles was a characteristic gross finding in 65 % of cases. In all cases the lungs revealed a pneumonitis noted as hypercellularity and thickening of the alveolar walls. Alveolar macrophages were present within the alveolar lumens. The latter lesion was regarded to be secondary to heart failure.

The clinical symptoms and pathological lesions observed are discussed and compared to those noted by various other workers both in South Africa and in other countries.

INTRODUCTION

Canine parvovirus (CPV) myocarditis was first recorded in 1979 in several countries including Australia[4], Canada[3], the United Kingdom[7] as well as the Republic of South Africa[11]. In 1978 in the United States, Pollock & Carmichael[7] observed parvovirus-like particles in the faeces of pups which were showing a severe enteritis and subsequently died. No myocardial lesions were seen in these pups. However, during 1980 Carpenter et al[2], working in the USA observed both the intestinal and myocardial form of CPV in puppies of the same litter. In South Africa both the enteritic and myocardial forms of CPV have been noted. According to Van Rensburg et al.[11], outbreaks of CPV in South Africa were usually associated with either the enteritic or cardiac forms as separate entities although in 2 outbreaks, myocarditis and enteritis were encountered in the same individual.

CPV infection was first reported in South Africa in December 1979[11], although cases of CPV myocarditis had occurred in February of the same year. Since then the incidence of CPV infection has increased greatly. The enteric form of CPV disease reached epidemic proportions throughout the country towards the end of 1980. There is as yet no report of the virus having been isolated in South Africa. In the United States, a strain of CPV (strain 916) was isolated from a dog with nonfatal enteritis and passaged for less than 4 times in canine and feline renal cells[1]. Carmichael et al.[1] have shown that CPV differs from the feline parvovirus and mink enteritis virus in its haemagglutinating ability[1]. This suggests that CPV is a distinct virus from the latter 2 viruses.

During the year 1979, 11 cases of parvovirus myocarditis were diagnosed on histopathological examination at the Pathology Department of the Veterinary Research Institute. This report describes the clinical symptoms and gross- and histopathological lesions observed in these cases and compares these findings with those reported by other workers both in South Africa and in other countries.

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HISTORY AND CLINICAL SIGNS

Eleven cases of CPV myocarditis arising from 8 litters were diagnosed during 1979. The first 2 cases in this series occurred in February in 2 Bulldog puppies arising from the same litter. These pups were 5 weeks of age. The remaining cases occurred at various intervals throughout the remainder of the year.

Details concerning the breed, litter size, age, number of pups that died and clinical findings are given in Table 1.

Death occurred suddenly in all the pups that died due to CPV myocarditis. Of the 11 pups, 10, varying in age from 3½ to 6 weeks, did not show any clinical signs prior to death. The eleventh pup developed a severe respiratory dyspnoea 12 h before death. This pup at 8 weeks of age was the oldest of the 11 puppies. Anaemia was noted in this same puppy, and a bloodsmear from this pup revealed polychromatophilia and anisocytosis. In 2 cases, diarrhoea was noted 4–7 days before death.

GROSS PATHOLOGY

Complete post-mortem examinations were not performed in all the 11 cases. The following findings were, however, observed. In pups from 4 out of 8 litters, anaemia was noted as a general paleness of the mucous membranes, organs and musculature. Pulmonary congestion and oedema were observed in all the pups. In 3 puppies the livers appeared congested and swollen.

In 3 of the 11 puppies, the hearts were paler than normal. Two of these also revealed dilatation of the ventricles, the one involving the left and the other the right ventricle. Pale white to gray streaks were noted on the epicardial surface of 1 pup.

On examining the formalin-fixed hearts submitted for histopathological examination, pale, white areas were noted in 7 hearts. These areas were extensive and arranged in a haphazard fashion over the endocardial or epicardial surfaces of the ventricles (Fig. 1 & 2). The apex and papillary muscles were the areas most commonly affected. The atria were not involved.

When sectioning the hearts, these white areas appeared as streaks or bands in the myocardium. Some areas extended over the entire thickness of the myocar-

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Table 1: CLINICAL AND OTHER FINDINGS IN 8 LITTERS OF PUPS

Litter No.	Case No.	Litter Size	Breed	Age	No. Dead	Cause of Death	Clinical Findings
I	—	8	Bulldog	3 w	2	Unknown	—
	1 & 2			5 w	2	CPV Myocarditis	Sudden death after suckling
II	—	5	Unknown	Unknown	3	Unknown	—
	3			3½ w	1	CPV myocarditis	Sudden death
III	—	5	Mixed breed	Unknown	3	Unknown	—
	4			4 w	1	CPV myocarditis	Sudden death; diarrhoea 1 w prior to death
IV	—	5	Mixed breed	2 w	2	Unknown	—
	5 & 6			4 w	2	CPV myocarditis	Sudden death
V	7 & 8	5	Bassett Hound	6 w	2	CPV myocarditis	Sudden death
VI	—	4	Bulldog	—	1	Unknown	—
	9			4 w	1	CPV myocarditis	Sudden death; diarrhoea 4 d prior to death
VII	10	4	Poodle	5 w	1	CPV myocarditis	Sudden death
VIII	11	Unknown	Bull Terrier	8 w	1	CPV myocarditis	Sudden death 12 h after onset of severe dyspnoea; weakness, shivering, & anaemia

d = days, h = hours, w = weeks, — = these pups were not examined either clinically or pathologically

dium (Fig. 1). Others formed a band below either the endocardial or epicardial surface and did not involve the entire thickness of the myocardium (Fig. 2). The affected areas felt firmer than the adjacent unaffected cardiac muscle, but this was difficult to confirm.

HISTOPATHOLOGY

In HE stained sections the hearts revealed a diffuse subacute to chronic fibrous myocarditis in all the cases. Single or groups of muscle fibres were replaced by fibrous tissue. In the subacute cases the affected tissue appeared cellular and was composed predominantly of fibroblasts and histiocytes, whilst in the chronic cases it was relatively acellular and composed of wavy bundles of collagen fibres (Fig. 3). In the subacute cases necrotic muscle fibres were present as homogeneous, eosinophilic, non-striated fragments interspersed amongst the inflammatory cells.

Round cells consisting predominantly of lymphocytes were seen to infiltrate the interstitium in all the hearts examined. These cells occurred either diffusely throughout the myocardium or as focal groups (Fig. 4).

Large, basophilic, intranuclear inclusions were seen in 10 of the 11 hearts. In 4 of these cases the inclusions were numerous and easy to find. The pup in which no inclusions were found was a chronic case. Inclusions were, however, present in the littermate of the latter pup. Some inclusions were oblong and extended over the entire length of the nucleus but not the entire width, so that a clear space could be seen at the edges of the inclusions. In the nuclei containing this type of inclusion the chromatin was marginated along the nuclear membrane (Fig. 5). The remainder of the inclusions filled the nucleus completely.

A moderate to severe pulmonary congestion and oedema was observed in all the puppies. Furthermore, the alveolar walls appeared thickened as well as hypercellular indicating a pneumonitis. Several alveolar macrophages were seen within the alveolar lumens (Fig. 6). A few lymphocytes, plasma cells and isolated neutrophils were noted scattered amongst the alveolar macrophages.

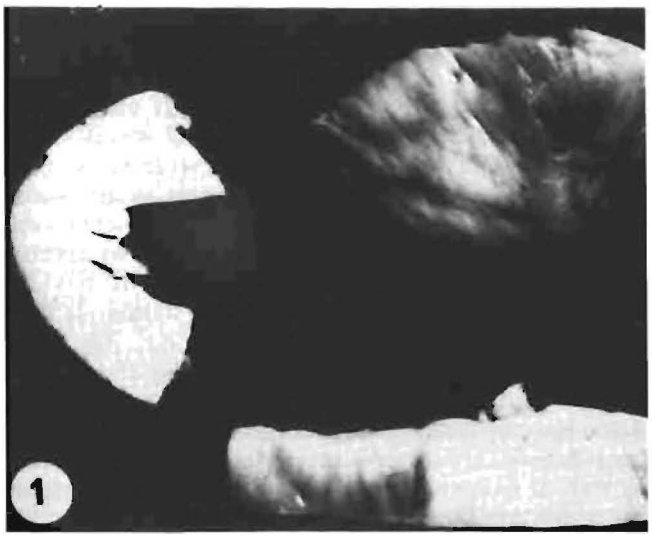
DISCUSSION

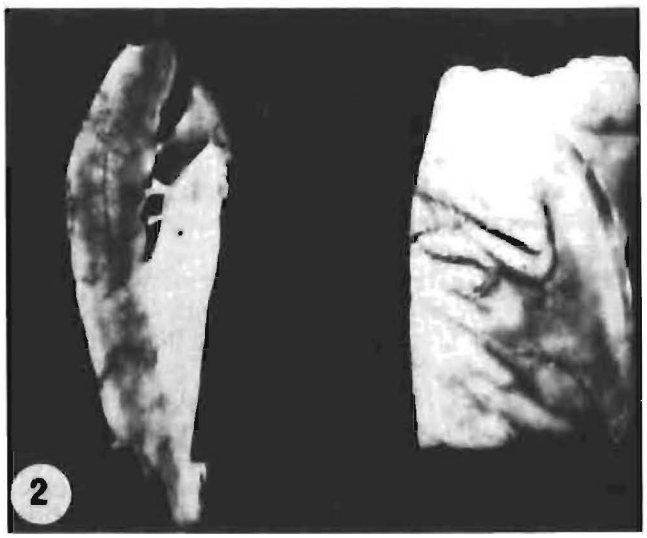
The age of the affected puppies in this report varied from 3½ to 8 weeks. CPV myocarditis has been reported to occur in young puppies from 2 to 12 weeks of age[1 3 6 8 11]. As parvoviruses require the replication of DNA for their own replication, they have a predilection for rapidly dividing cells such as intestinal epithelium, bone marrow and lymphoid organs[3 9]. The predilection of CPV for myocardial cells may be related to this prerequisite of parvoviruses because, according to Robinson et al[9], 2–4 % of myocardial cells undergo division in newborn puppies up to the age of 15 days, after which the rate of division declines steadily[9].

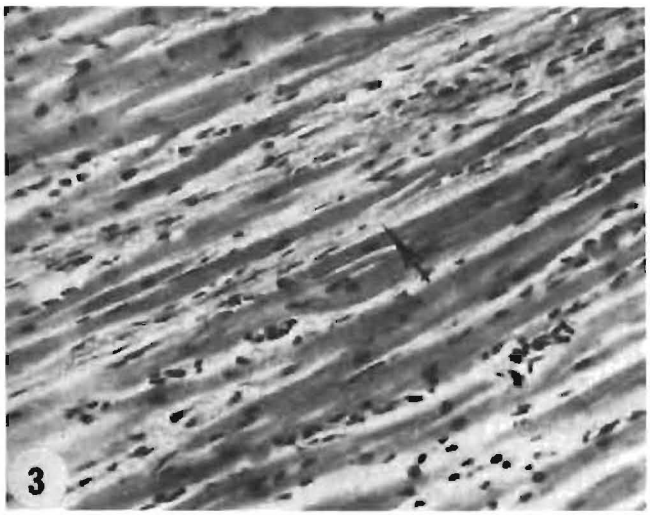
A constant finding in all the puppies was sudden death. This finding has been reported by various authors from several countries[3 4 6 10 11]. The pup that was 8 weeks old developed a severe respiratory dyspnoea 12 hours before death. Robinson et al[9] reported that CPV myocarditis can present as 1 of 2 clinical syndromes, viz., sudden death without any prior symptoms in puppies 3–8 weeks of age or the development of severe dyspnoea 1–24 hours before death in puppies 8 weeks of age or older. The clinical syndromes noted in the 11 pups in this series agreed with these findings.

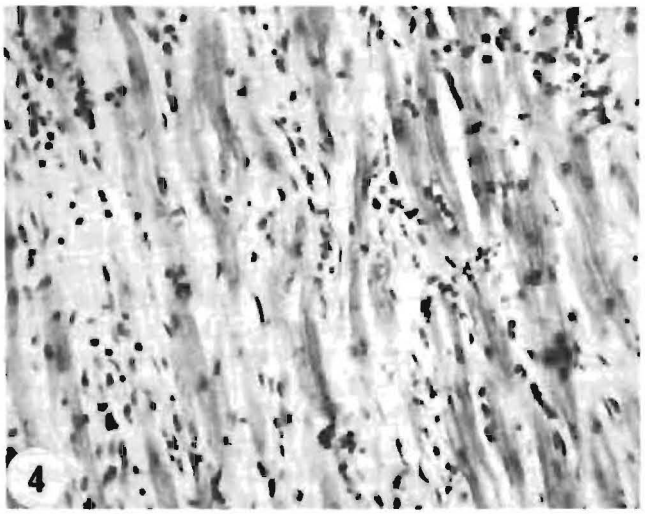
Two of the pups developed a diarrhoea 4–7 days prior to death. The cause of the diarrhoea in these 2 pups was not determined. CPV myocarditis has been reported to occur in conjunction with the enteritic form or in puppies which have apparently recovered from the enteritic form[2 7]. Hence, it is possible that these 2 puppies developed the enteritic form and later succumbed to the myocardial form of the disease.

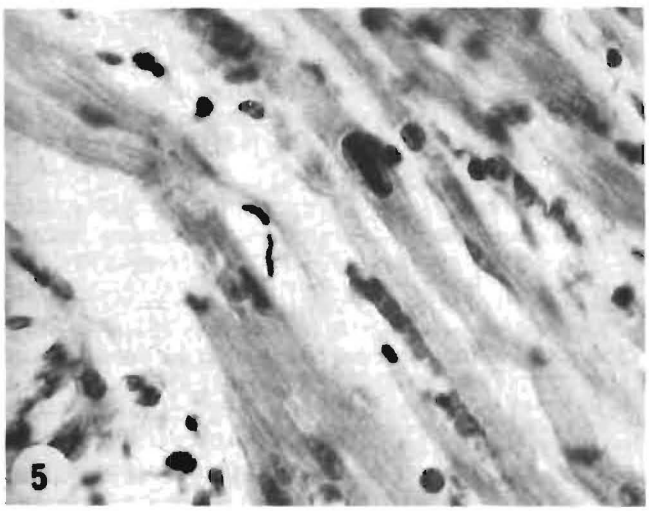
The pale appearance of the muscles and mucous











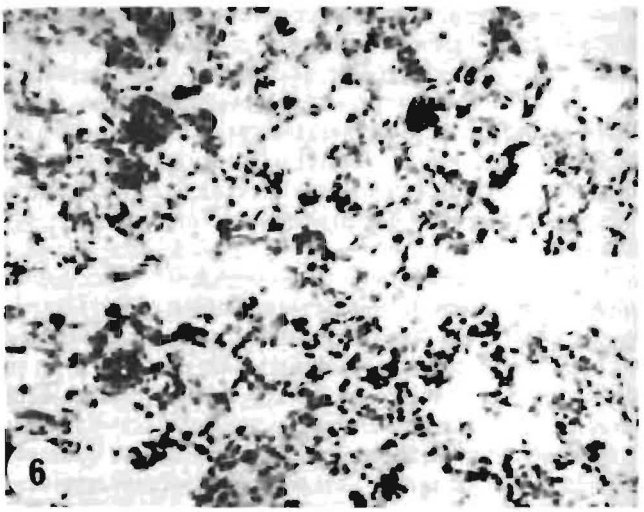


Fig. 1 Left: Fibrosis involving the entire thickness of myocardium Right (2 specimens): Patchy fibrosis as seen on epicardial surface of formalin-fixed sections of heart.

Fig. 2 Left: Band of fibrosis subendocardially, involving the entire papillary muscle in a formalin-fixed section of heart. Right: Patchy white areas of fibrosis on the epicardial surface.

Fig. 3 Mature wavy collagenous tissue replacing groups of muscle fibres (arrow). HE X200

Fig. 4 Scattered areas of fibrosis. Note also infiltration of round cells (lymphocytes) into interstitium. HE X200

Fig. 5 Large oblong, basophilic intranuclear inclusion extending along the entire length of the nucleus. HE X500

Fig. 6 Congestion and thickening of the alveolar walls. Alveolar macrophages can be seen within the alveolar lumens. HE X200

membranes in 4 of the pups suggests an anaemia. In one pup, the blood smear picture supports this finding. Unfortunately however, no red cell counts or haemoglobin values were determined to conclusively support this finding. Van Rensburg et al[11] noted anaemia in puppies with CPV myocarditis, but they did not make any mention of red cell counts or haemoglobin values. Jubb & Kennedy[5] reported that anaemia may be a feature of feline panleucopaenia.

Ventricular dilatation was observed in 2 cases. However, as 9 of the hearts were only examined after they had been fixed in formalin, it is possible that ventricular dilatation was present in more of the pups. Out of a series of 11 cases, Hayes et al.[3] reported right ventricular dilatation in 3 cases and bilateral ventricular dilatation in 1 case. Kelly & Atwell[6] and Huxtable, et al.[4] observed slight enlargement of the hearts of puppies with CPV myocarditis due mainly to dilatation of the left ventricle.

White streaks or bands of fibrosis were noted in the ventricles of 65 % of cases (7 puppies). These lesions were easily overlooked on cursory examination but were quite distinct when the hearts were examined carefully. According to Hayes et al[3] areas of fibrosis were observed at gross post-mortem examination in only 9 % of cases. On the other hand, Robinson et al[9] observed similar lesions in the majority of their cases but did not mention the exact number.

In this report, CPV myocarditis diagnosis was based on the typical histopathological appearance of the heart, viz., a diffuse fibrous myocarditis and the presence of large, basophilic, intranuclear inclusions in the cardiac myofibres. By the use of electron microscopy, several workers have shown that these inclusions consist of aggregates of 20–22 nm in diameter sphericle viral particles that are typical for parvoviruses[2 3 9]. The fibrous myocarditis was observed in all the puppies whilst inclusions were seen in 10 of the 11 pups (90 %). Carpenter et al[2] reported fibrous myocarditis to be a constant finding but found inclusions in only 50 % of cases. According to these same authors, inclusions are difficult to find or can be absent in older puppies with more chronic lesions[2]. The pup in this series in which no inclusions could be found was a chronic case. Similarly, Robinson et al[9] observed intranuclear inclusions in all pups 3–8 weeks old but did not observe them in the more chronic cases in pups 8 weeks or older. In only 4 pups (36 %) were the inclusions numerous and easy to find.

The sudden death of puppies with CPV myocarditis could be ascribed to the extensive nature of the fibrosis in the hearts. By studying electrocardiagrams of pups with CPV myocardiitis, Carpenter et al[2] were able to show that the sudden death was due to blockage of impulse conduction.

Pulmonary congestion and oedema was a constant feature in all the pups. This finding has been reported by various authors[1 6 8 9 11]. Robinson et al[9] noted a translucent zone of oedema about 2,5 mm wide around the bronchial and vascular trees. Such a lesion was not noted in the puppies dealt with in this report and has not been described by other authors. Thickening and hypercellularity of the alveolar walls, as observed in this report, has been reported on several occasions[2 3 9]. This lesion can be regarded as a pneumonitis. Carpenter et al[2] referred to such a lesion as an interstitial viral pneumonia, as they noted inclusions in what were thought to be alveolar macrophages. These authors were of the opinion that the number of cells they observed in the alveolar septa were more numerous than can be expected from heart failure alone[2]. The presence of alveolar macrophages within the alveolar lumens together with a pulmonary congestion and oedema indicate that the lung lesions could also be due to heart failure. This view is supported by Robinson et al[9] as well as Carpenter et al[2]. As the pulmonary lesions are a constant finding, Carpenter et al[2] coined the term "cardiopulmonary" to describe the myocardial form of CPV infection. This appears to be a good descriptive term to use, as it indicates the organs that are pathologically involved.

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PLASMA CORTISOL LEVELS IN PIGS SUSCEPTIBLE AND RESISTANT TO MALIGNANT HYPERTHERMIA

G. MITCHELL* and J.J.A. HEFFRON**

ABSTRACT: Mitchell G.; Heffron J.J.A. **Plasma cortisol levels in pigs susceptible and resistant to malignant hyperthermia.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 109–112 (En) Department of Physiology, University of the Witwatersrand Medical School, Hospital Street, 2001 Johannesburg, Rep. of South Africa.

Plasma cortisol levels were measured in Landrace pigs susceptible to malignant hyperthermia (MH), Landrace pigs resistant to MH and in Largewhite pigs, during growth halothane anaesthesia and after injections of Synachten. From 3 to 6 months of age mean cortisol levels in susceptible Landrace were 193,2 ± 49,7 nmol/ℓ, in resistant Landrace 220,8 ± 24,8 nmol/ℓ and in Largewhite 278,8 ± 13,8 nmol/ℓ. These values were not significantly different. After injection of 1 mg Synachten intravenously, plasma cortisol levels did not increase significantly in susceptible pigs. In resistant Landrace pigs and Largewhite pigs the plasma levels after injection were significantly greater than pre-injection levels ($P < 0,05$) and significantly different from susceptible Landrace levels. During halothane anaesthesia, cortisol levels rose more in resistant pigs than in susceptible pigs, but differences were not significant.

These results suggest that susceptible pigs have a decreased ability to secrete cortisol during stress.

INTRODUCTION

Although the primary lesion in both porcine malignant hyperthermia (MH) and MH in man is considered to be identical[1] some major differences exist between the 2 syndromes. For example, there is a good correlation between clinical and subclinical myopathy and susceptibility to MH in man[2] whereas in pigs there is little evidence for a similar correlation. On the other hand, in pigs there is considerable data to show that hormones play a role in the development of the syndrome[3 4 5]. In man, few hormonal aberrations have been described although steroids are used in most treatments of established MH in man[6].

In the porcine syndrome, thyroid hormones and adrenal hormones have been extensively investigated, especially T_3[7] and catecholamines[8]. The role of adrenal steroids, particularly the glucocorticoid cortisol, in the syndrome is more controversial. The first reports[9] of the pale soft exudative (PSE) pork syndrome, which is allied to the porcine MH syndrome, noted that the adrenocrticotrophic hormone (ACTH) content of the anterior lobe of the pituitary was reduced in stress-susceptible pigs. Further, the rise of plasma lactate and muscle rigidity characteristic of the syndrome could be alleviated by injection of 50 mg hydrocortisone before exercise[9]. From these results, Ludvigsen[9] drew the conclusion that stress susceptibility was associated with adrenal insufficiency. Lister et al.[10] have refuted this view. Conversely, it has been shown that heat stress produces an increase in 17-hydroxycorticosteroids, the greatest increase occurring in animals producing poor quality meat[11]. Further, Marple et al. have shown both a fall in cortisol levels during heat stress[12] and an increase[13] but have suggested that because ACTH levels are high when cortisol levels are low, there is increased metabolism of cortisol in stress-susceptible pigs[12]. A further complication is produced by the therapeutic use of steroids. Ellis et al.[6] showed reversal of a nitrous-oxide-induced MH in man by dexamethasone and that *in vitro*, hydrocortisone abolished halothane-induced muscle contraction. Hall et al., however, have not been able to repeat this because large doses of methyl-prednisolone failed to change the course of hyperthermia in pigs[14].

In order to try and resolve the controversy, we have measured cortisol levels in stress-susceptible and stress-resistant pigs during growth and halothane anaesthesia and in response to injection of ACTH.

METHOD

Forty-two pigs were used in this study. Thirteen of the pigs were Largewhites which had no genetic or other history of susceptibility to any of the porcine stress syndromes. Twenty-nine of the pigs were obtained from a herd with a high incidence of MH susceptibility. All the pigs entered the trial at the age of 8 weeks, were raised identically and fed a standard ration.

Blood for determination of plasma cortisol levels was obtained by jugular puncture at the thoracic inlet, between 08h00 and 11h00. Plasma cortisol was determined using the Cortipac (Radiochemicals, Amersham) radioimmunoassay kit. Each plasma sample was divided into 2 aliquots. The mean cortisol value obtained from each of the aliquots was taken as the plasma cortisol level for that sample.

In the first series of experiments, plasma cortisol was measured in 6 Largewhite pigs at 4, 5 and 6 months of age and in 10 Landrace pigs at 3, 4, 5 and 6 months of age, to assess changes in plasma cortisol during growth.

In the second series of experiments, 1 mg Synachten (Ciba) was injected intravenously into 2 Largewhite pigs and 8 Landrace pigs. A blood sample was taken before injection and at 30 and 60 min after the injection to assess the adreno-cortical response to Synachten.

In the third series of experiments, 6 Largewhite pigs and 10 Landrace pigs were anaesthetized using halothane (Fluothane, ICI). A blood sample was taken before exposure to halothane and at approximately 5 min intervals during anaesthesia. Anaesthesia was discontinued after 15 min in resistant pigs. In pigs reacting to halothane, halothane anaesthesia was continued until death.

Halothane, for this experiment and to determine susceptibility to MH, was administered using a close-fitting face mask, a semi-closed circuit system and a Fluotec Mk III vaporizer. The pigs were induced with 10 % halothane in oxygen at a flow rate of 1,5 to 2,0 ℓ/min. Within 1 to 2 min the animals became comatose

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and the halothane concentration was reduced to 5 %. After a further 1 to 2 min the halothane concentration was reduced to 3 % for the duration of anaesthesia.

An unpaired one-tailed student's t-test was used for statistical analysis of the results.

RESULTS

Halothane challenge revealed that 9 of the Landrace pigs were susceptible to halothane-induced MH. The 13 Largewhite pigs and the other 20 Landrace pigs survived anaesthesia and were considered to be MH resistant.

Cortisol levels during growth

Table 1 summarises the cortisol levels in the plasma of the 3 groups of pigs. During growth the 3 MH susceptible pigs had consistently lower cortisol levels than either MH resistant Landrace pigs or Largewhite pigs. However, we could not show significant differences between the cortisol levels of the 3 groups of pigs.

Cortisol levels after Synachten

Table 2 shows that susceptible pigs have consistently lower plasma cortisol than either resistant Landrace or Largewhite pigs. Table 2 also shows that after injection of Synachten plasma cortisol increased in all 3 classes of pigs. In none of them did the 30 min value differ significantly from the 60 min value. However, the 30 min value differed significantly from pre-injection values in the Largewhite group ($P < 0,005$), and the 60 min value differed significantly from pre-injection levels in both resistant Landrace ($P < 0,025$) and Largewhite pigs ($P < 0,001$) but not in susceptible pigs. The rate of increase of plasma cortisol was 13,2 nmol/min for susceptible pigs, 22,1 nmol/min for resistant Landrace and 25,1 nmol/min for Largewhite pigs. Further, Table 2 shows that post-injection increase in plasma cortisol in both resistant Landrace and Largewhite pigs is significantly greater than the post-injection increase in susceptible pigs ($P < 0,05$).

Cortisol levels after halothane

The effect of halothane on plasma cortisol levels is shown in Table 3. Although we could not show statistically significant differences between the 3 groups of pigs after halothane anaesthesia, susceptible pigs had lower absolute levels of cortisol than did either of the other 2 groups. The rate of increase of cortisol after halothane was also lowest in susceptible pigs (38,6 nmol/min), whereas in resistant Landrace pigs it was 69,0 nmol/min and in Largewhite pigs 60,7 nmol/min for the first 5 min. The mean cortisol levels in all post-

Table 1: PLASMA CORTISOL LEVELS DURING GROWTH IN LANDRACE AND LARGEWHITE PIGS.

		No. of pigs	Cortisol nmol/ℓ				
			3 months	4 months	5 months	6 months	Mean
Landrace	Susceptible	3	132,5 ± 30,4	143,5 ± 19,3	74,5 ± 16,6	422,3 ± 162,8	193,2 ± 49,7
	Resistant	7	143,5 ± 22,1	184,9 ± 24,8	149,0 ± 49,7	408,5 ± 74,5	220,8 ± 24,8
Largewhite		6	—	212,5 ± 19,3	311,9 ± 138,0	314,6 ± 41,4	289,8 ± 13,8

Table 2: PLASMA CORTISOL LEVELS IN LANDRACE AND LARGEWHITE PIGS AFTER INJECTION OF 1 mg SYNACHTEN INTRAVENOUSLY

		No. of pigs	Cortisol nmol/ℓ			
			0 min	30 min	60 min	mean post-injection
Landrace	Susceptible	3	182,2 ± 96,6	483,0 ± 298,1	579,6 ± 281,5	532,7 ± 140,8
	Resistant	5	331,2 ± 157,3	761,8 ± 231,8	990,8 ± 237,4	874,9 ± 99,4
Largewhite		2	209,8 ± 8,3	979,8 ± 127,0	966,0 ± 58,0	974,3 ± 38,6

Table 3: PLASMA CORTISOL LEVELS IN LANDRACE AND LARGEWHITE PIGS AFTER EXPOSURE TO HALOTHANE ANAESTHESIA

			Cortisol nmol/ℓ					
				post-halothane				
		No. of pigs	pre-halothane	5 min	10 min	15 min	20 min	Mean post-halothane
Landrace	Susceptible	3	220,8 ± 49,7	458,2 ± 104,9	452,6*	477,5*	985,3*	546,5 ± 99,5
	Resistant	7	292,6 ± 82,8	640,3 ± 93,8	734,2 ± 184,9	770,0 ± 35,9	844,6*	698,3 ± 46,9
Largewhite		7	342,2 ± 49,7	645,8 ± 41,4	—	—	—	645,8 ± 52,4

*data from 1 pig.

halothane blood samples were also not significantly different. Table 3 shows, however, that the cortisol levels of susceptible pigs before exposure to halothane (220,8 ± 49,7 nmol/ℓ) were significantly less than the prehalothane cortisol levels for Largewhite pigs (342,2 ± 49,7 nmol/ℓ $P < 0,05$), confirming the tendency shown in Table 1.

DISCUSSION

The controversy surrounding a role for cotrisol in the porcine stress syndromes rests on the one hand on findings showing that cortisol given prophylactically prevents a marked increase in plasma lactate and muscle rigidity in stress-susceptible pigs[9]. *In vitro,* cortisol will also inhibit halothane-induced muscle contraction[6]. Further ACTH levels in the anterior pituitary of stress-susceptible pigs are low[9]. These observations suggest that a pituitary or pituitary-adrenocortical insufficiency exists and that decreased cortisol may precipitate muscle rigidity. On the other hand, stress-susceptible pigs have high plasma levels of ACTH[12] and high rates of production and utilization of cortisol[12]. Synachten injections have also elicited a similar increase in plasma cortisol in both stress-resistant and susceptible pigs[10]. Our results show that plasma cortisol levels in stress-susceptible pigs are consistently lower than in stress-resistant pigs and that both the rate of increase and the total increase of cortisol after injections of Synachten are lower in stress-susceptible pigs. We were not able to show differences in values that were consistently significant, however, probably because of both the wide range of values found in susceptible pigs as well as the small sample size.

The consistently lower levels of cortisol in stress-susceptible pigs can be a result of exhaustion of the adrenal cortex and thus decreased secretion, increased metabolism or utilization of cortisol, impaired cortisol biosynthesis or reduced stimulation by ACTH of the adrenal cortex. The lower levels during growth could easily be a result of rapid metabolism or utilization. However, in our view this is unlikely. Stress-susceptible pigs have been shown to have lower urinary steroid secretion than resistant pigs which argues decreased secretion of cortisol rather than increased use or metabolism[4]. Further support for the idea that decreased secretion is responsible for low plasma cortisol is provided by our data on cortisol levels after Synachten and halothane. After injections of Synachten, cortisol levels did not increase as rapidly or to the same extent in susceptible pigs as in resistant pigs. The rate of increase in susceptible pigs was approximately half that in resistant pigs and the post-injection cortisol levels were significantly less in susceptible pigs.

A similar response was noted after halothane anaesthesia. In normal humans, halothane anaesthesia stimulates adrenocortical function[15] and both plasma ACTH and plasma-free cortisol levels increase after halothane[16]. If resistant pigs can be regarded as normal, our data suggest that stress-susceptible pigs have both a slower rate of increase in plasma cortisol and a smaller average increase of plasma cortisol after exposure to halothane. However, these responses were not significantly different between the 3 groups of pigs. Further, the rate of increase in cortisol after halothane was threefold higher than the rate of increase after Synachten which suggests that in susceptible pigs the adrenal cortex cannot be exhausted and it must be synthesizing cortisol. Another implication of our data is that the adrenal cortex cell receptors do not properly interact with ACTH. This defect could explain the high plasma ACTH, the low pituitary ACTH, low plasma cortisol and low urinary excretion of steroid metabolites which are characteristic of stress-susceptible pigs.

Although our data suggest decreased secretion of cortisol, they also show that decreased secretion is only uncovered by stress. During growth, cortisol levels are similar in the 3 groups of pigs, although susceptible pigs have consistently lower levels of plasma cortisol. Further, even if these differences could be shown to be statistically significant in a larger sample of pigs, it seems unlikely that 25–80 nmol/ℓ difference in cortisol levels would have profound physiological effects.

In summary, our results suggest that in non-stressful situations cortisol secretion in both susceptible and resistant pigs is similar. However, the ability of stress-susceptible pigs to secrete cortisol during stress is reduced. It seems unlikely that the lower cortisol levels observed in susceptible pigs is due to reduced pituitary secretion of ACTH since injections of synthetic ACTH (Synachten) have less effect on plasma cortisol levels in susceptible pigs than in resistant pigs. Halothane, which also stimulates ACTH secretion[15], also causes less of a response in susceptible than in resistant pigs. In view of the well-established membrane-stabilizing effect of cortisol and its role in the regulation of glycolysis and glycogenolysis, a reduced secretion of cortisol may play a contributory role in the development of porcine PSE and MH.

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BOOK REVIEW BOEKRESENSIE

A GUIDE TO CANINE ORTHOPAEDIC SURGERY

H.R. DENNY
Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Melbourne 1980 pp 184 Figs 365 Price R16,80
(ISBN 0-632-00579-3)

In the Preface of this book the author describes his goal as follows: ". . . It is hoped that this format will allow for rapid reference". He also states that the main disadvantage of a book of this type is that the conditions cannot be discussed in depth. This is evident when reading the book, particularly when one seeks information about a specific condition or surgical procedure. Although the conditions are not described in great detail, very few orthopaedic conditions of the dog have been omitted and the field has been thoroughly covered.

The first two chapters deal with the classification, healing, complications and treatment of fractures in general. Chapter Three describes the surgical conditions of the skull and spine. Chapters Four and Five concern the various conditions affecting the forelimb and hindlimb. Appropriate references are provided at the end of each chapter for further reading.

The operative techniques described are the latest techniques used in canine orthopaedics. The book is well illustrated with clear and simple line drawings which enhances the value of the book. In spite of the fact that many conditions and procedures are dealt with superficially, it can be highly recommended to practitioners and students as a "rapid reference" book.

D.G. STEYN

A SUCCESSFUL HERD MASTITIS CONTROL SCHEME IN NATAL†

R.W. Bryson* and W.B. Hobbs**

ABSTRACT: Bryson R.W., Hobbs W.B. **A successful herd mastitis control scheme in Natal.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 113–117 (En) Allerton Laboratory, Private Bag X 9005, 3200 Pietermaritzburg, Natal, Republic of South Africa.

Somatic cell counting combined with bacterial identification was successfully used as a method of reducing dairy-herd mastitis problems in Natal. Application of the recommended control measures resulted in an average increase in daily production of up to 2,8 ℓ of milk per cow per day, improvement in quality and reduction in the prevalence of mastitis.

INTRODUCTION, HISTORY AND OBJECTIVES

More than 400 fresh-milk producers in Natal are registered with the Durban City Health Department to supply milk for consumption within the city. The Veterinary Hygiene Section of the Health Department exercises control over the premises from which the milk originates, the suitability and cleanliness of the equipment used in its production, storage, transportation, pasteurisation, bottling, etc. It is particularly interested, of course, in the hygienic quality of the product at all stages until it reaches the consumer. As part of this latter requirement a programme of sampling and testing of individual herd milks has been in progress for many years. In practice, each producer's milk is sampled 8–10 times for this purpose.

During the second half of 1973 acquisition of a Coulter Counter made it possible to carry out accurate somatic cell counts (SCC) on these herd milk samples. Farmers were informed of the significance of various levels of milk SCC and were advised by means of literature, film shows and lectures on mastitis control measures. The aim of informing producers of their bulk milk SCC on a regular basis was to make them aware of the extent of the subclinical mastitis problem and to promote an interest in improving herd udder health as demonstrated objectively by a falling cell count. Naturally, improved milk quality and quantity would be the end result.

Following the first 6 months of cell counting, it was found that some 22 % of herds had a mean SCC in excess of one million cells per mℓ and it soon became apparent that all herds fell into one of 4 groups:[1]

1. SCC low, indicating a negligible mastitis problem and good herd management.
2. SCC initially moderate to high where the producer was both interested and successfull in improving herd udder health, as evidenced by a substantial drop in SCC.
3. SCC initially moderate to high where the producer showed an interest in improving the situation but had little or no success despite his efforts.
4. SCC moderate to high where the producer showed little or no interest in improving udder health. These herds almost invariably showed a rising SCC.

In September 1977 legislation was gazetted making illegal the sale of milk containing more than one million cells per mℓ. This legislation enabled the Durban City Health Department to exert greater pressure on the producers who had previously shown little or no inclination to control mastitis within their herds. These producers and those of Group 3 above were given assistance such as advice on milking technique, milking-machine operation, parlour hygiene, teat dipping, therapy, etc. and, on request, their machines were checked for efficiency of operation. However, it was suggested to many of these people that they required additional laboratory assistance over a period in order to identify infected cows, determine causative organisms and generally to enable a complete assessment of the type and extent of the herd problem to be made so that effective advice could be given. For this reason the Allerton Veterinary Investigation Centre decided to launch a pilot mastitis control scheme during 1977.

Farmers are suspicious of anything new and initially no charge was made for the service. It was also decided to work only on herds where assistance had been specifically requested by the owner and where prior evaluation showed that progress could be made. Therefore the first step in each herd involved an on-farm inspection to check facilities available, standard of hygiene maintained, quality of management and whether or not a viable farming system was in operation. The salient points were:

(i) Milking must take place in hygienic surroundings
(ii) Milking equipment must be efficient (this involves a six-monthly or annual visit by the serviceman)
(iii) The use of good milking technique
(iv) Elimination of stress factors (e.g. rough handling, cold, wet or muddy conditions, etc.)
(v) Prompt diagnosis of clinical cases
(vi) Efficient treatment of clinical cases
(vii) Keeping of complete records of clinical cases
(viii) Culling of chronic cases
(ix) Use of a good teat dip at each milking
(x) Attention to health of skin of teats
(xi) Possible treatment of all quarters at drying-off with the correct long-acting antibiotic, depending on the extent of infection
(xii) The use of uncontaminated water in the dairy – samples are taken for a presumptive coliform test.

All short-term mastitis control is aimed at:

(a) the elimination from the herd of chronic and intractable cases
(b) the prevention of as many new cases as possible
(c) The curing of as many affected quarters as possible by
  (i) the use of antibiotics only in clinical cases and at drying off

†Paper delivered at the Annual Congress of the Natal branch of SAVA, April 1980.

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(ii) promoting spontaneous or "self-cure" of subclinical cases.

The farmer was told of factors found to be deficient and he was asked to re-apply to enter the scheme when improvements had been made. It was stressed that the scheme was voluntary, that the Division of Veterinary Services was making the initial investigation and that veterinary practitioners, who were kept fully informed, could take part and possibly would take over the control at a later stage. In fact, these veterinarians were highly co-operative from the outset and have all along unreservedly recommended that their dairying clients join the scheme.

The objectives of the scheme are:

(a) To identify the cows responsible for the high SCC of the bulk milk
(b) To identify the organisms responsible – this has a considerable influence on the prognosis and handling of the herd problem
(c) To carry out antibiograms enabling the use of the most effective antibacterial remedies
(d) To recommend measures to cure or otherwise eliminate these infections and to limit further spread to healthy quarters
(e) To lower the bulk milk cell count to at least legal levels but preferably to below 400 000 cells per mℓ

The overall effect of these measures would be to decrease both clinical and subclinical mastitis, reduce wastage of both milk and cows, reduce treatment cost and increase milk production and the profitability of dairying.

PROCEDURE

This was described in detail at the 1st South African Mastitis Symposium in 1978[2].

To summarize, farmers are provided with sterile sample bottles and are instructed on a simple method of taking milk directly from each cow. A composite sample is taken first and repeated monthly until 3 consecutive samples have been taken. On those samples showing a SCC in excess of 750 000 per mℓ, bacterial identification is carried out followed by antibiograms on selected types of pathogenic bacteria.

A second farm visit is made after the results of these 3 tests have been studied to discuss progress with the farmer and to decide which of the chronic cases now identified should be treated or culled. A decision on this will be influenced by the types of bacteria involved and the extent of the chronic infection. The latter is assessed by quarter-sampling cows which the farmer wishes to retain.

RESULTS

The extent of success of a mastitis control programme in a dairy herd can be measured in several ways, viz. lowered bulk milk SCC, fewer low-lactose producing cows, reduction in number of clinical mastitis cases and ultimately increased production of butterfat and milk. Increased milk and butterfat production is difficult to measure except in a controlled trial as other variables, notably plane of nutrition and management, exert a major influence.

Tables 1 and 2 show details of some of the herds that have been involved in the scheme prior to April 1979 and which fall under Durban Cith Health Department for sampling and cell-counting purposes. Table 1 shows the date of entry of selected herds into the control scheme together with a six-monthly arithmetic mean bulk milk SCC prior and subsequent to entering the scheme. Table 2 shows the percentage of cows in the same herds producing low-lactose milk. The herd numbers correspond in each table.

Figure 1 illustrates in histographic form the distribution of 272 herds into SCC groups, the herds included being those that were supplying milk to Durban in 1973 when cell-counting commenced and are supplying milk at the present time. Approximately 25 % of these herds have been included in the scheme for variable lengths of time.

It is not possible to record the long-term results in each herd, but the experiences of a few selected herds are described where management and co-operation were above average:

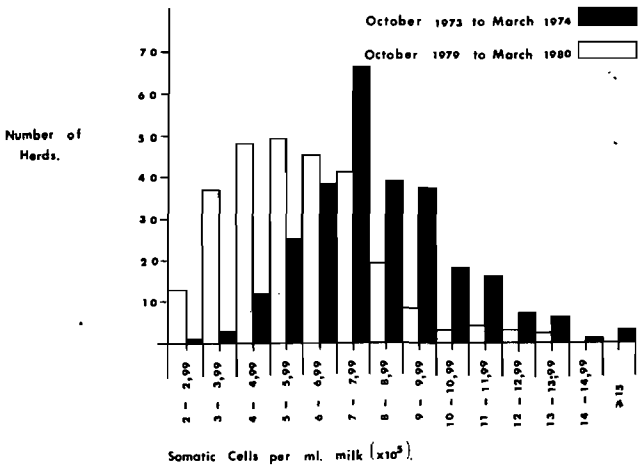

Fig.1 Distribution of 272 herds into cell count groups.

Herd No. 2: 85 cows. Entered scheme 14 February 1977

During 1976–77, clinical cases were occurring at the rate 15 per month, antibiotics for treatment costing R2 000 /a. Culling started on entry to the scheme when infection was found to be extensive, mainly *Staphylococcus aureus*.

From 1977 to 1980, 54 cows were culled for chronic mastitis. By 1980 the herd had been stabilised at 85 cows. The financial turnover from 120 cows was R51 648 in 1976. In 1979, 85 cows showed increase turnover to R70 000. Average daily production per cow had increased by 2,5 kg, giving an increased turnover of R15 512. To this must be added saving on antibiotics, veterinary fees and discarded milk to show increased turnover of R17 000 /a.

Clinical mastitis has been reduced to negligible proportions:

During Jan./Feb. 1980, 3 cases were recorded. All were easily cured and were probably of streptococcal aetiology.

Six-monthly mean SCC to March 1976: 1 638 000
Six-monthly mean SCC to March 1980: 731 000

Herd No. 11: 96 cows. Entered scheme 6 November 1977

Not as well managed a herd as No. 2 and had the

Table 1: MEAN MONTHLY BULK MILK SCC PER ml (THOUSANDS)

Herd No.	No. of cows	Date of entry into scheme	Six month period ending: 9/77	3/78	9/78	3/79	9/79	3/80
2	100	16.03.78	1226	990	822	655	673	731
4	154	19.02.79	616	822	466	728	582	303
7	78	30.01.79	830	1030	825	632	643	506
8	100	15.11.79	871	1367	825	782	633	601
8a	80	15.11.79	738	1101	982	876	605	400
11	96	6.12.77	1458	1005	1010	710	550	714
12	60	21.01.77	461	513	327	329	367	347
16	150	20.01.77		1334	1320	1010	1007	692
17	65	7.09.78	1189	1142	1044	834	801	692
18	112	30.08.78	1205	952	849	564	734	397
22	75	8.08.79	798	1210	1112	690	434	477
26	121	11.04.79	886	990	753	662	572	446
29	40	9.05.78	494	725	700	861	909	846
32	137	14.03.78	869	899	664	564	432	416
33	42	3.03.78	1784	1970	1702	1289	1035	584
37	144	11.09.78	995	846	787	645	652	332
38	84	2.11.76	888	566	828	793	857	537
44	215	6.12.77	1079	938	865	852	817	751
45	137	29.09.78	1488	1694	1141	1008	863	949
46	264	14.12.78	2252	1161	709	396	427	348
51	89	31.01.79	3945	1505	993	588	873	742
52	90	20.03.79	1147	1213	1256	1017	1233	895
59	40	29.08.78	613	695	788	591	506	344
60	50	21.11.79	885	841	1170	813	713	868
65	160	24.04.79	841	867	854	632	644	454
66	126	14.11.79	1115	1144	861	1078	852	730
67	107	2.11.78	882	1173	1154	1105	853	864
71a	67	2.06.77	1848	1226	701	723	656	476
75	112	19.07.78	1123	998	969	684	799	722

Table 2: PERCENTAGE OF COWS SHOWING LOW LACTOSE ON MONTHLY TESTS: 1978/79

Herd No.	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
2	1978	1	10	7	13	20	19	15	12	10	4	19	
	1979	2	2	3	0	7	0	0	3	0	3	7	3
4	1978	25	54	60	47	37	39	26	13	22	25	9	16
	1979	35	10	48	43	21	29	24	15	25	12	19	10
7	1978	15	10	31	40	54	19	15	34	21	5	7	5
	1979	12	6	8	10	9	10	7	1	0	6	24	2
8	1978	20	27	42	38	32	39	22	26	20	13	13	12
	1979	9	9	9	18	15	4	4	3	6	8	6	15
8a	1978	24	48	37	38	36	20	6	31	35	25	5	47
	1979	20	9	33	11	7	6	0	4	6	2	2	12
11	1978	42	43	48	59	63	35	16	26	30	39	11	10
	1979	12	5	12	21	21	5	11	11	3	7	8	14
12	1978		8	2	51	14	23	21	23	26	27	22	24
	1979	26	28	20	17								
18	1978	33	10	18	58	39	18	14	12	7	—	12	15
	1979	18	17	18	9	3	3	1	0	0	3	4	16
26	1978	40	57	47	66	51	26	41	21	45			42
	1979	22	12	10	30	18	12	20		13	9	27	12
37	1978	25	29	19	55	28	28	17	21	20	28	30	23
	1979	27	26	11	17	8	1	7	9	5	7	12	4
44	1978	39	67	34	68	62	24	53	28	48	45	33	83
	1979	33	19	33	25	22	14	44	10	8	9	9	24
60	1978	3	22	16	29	32	5	28	9	19	21	13	19
	1979	11	17	14	21	21	5	15	7	5	2	8	3
65	1978	7	25	13	49	36	7	23	33	21	12	3	5
	1979	19	3	4	12	6	9	8	5	2	2	13	7
66	1978	28	38	34	53	47	55	29	7	13	8	18	5
	1979	10	11	19	28	26	12	18	9	12	6	3	7

highest prevalence of infection of all the herds. Milking machine and hygienic practices were not up to standard but were corrected. Owner not always present at milking.

Some 60% of cows showed *S.aureus* infection at first test after the private veterinary surgeon had referred the herd to us in despair. Drastic culling was financially not feasible and owner had tried a variety of remedies. Thirty cows have been culled to date. Another 15 will eventually be culled and are presently milked separately with the milk being used on the farm for pigs, calves, etc. This has resulted in a loss of 45 × R300 (½) = R13 500. Many cows would have been routinely culled.

Average daily yield has increased by 2,5 ℓ/d, giving increased production of 96 × 2,5 × 20c × 365 = R17 520 /a. In addition, milk disposal was reduced by R1 000, mastitis remedies by R800 giving a total annual saving of R19 320. No great expense was incurred on improving milking practice.

Six-monthly mean SCC to March 1977: 1 458 000
Six-monthly mean SCC to March 1980: 714 000

The farmer freely acknowledges that the mastitis control scheme saved him from complete disaster.

Herd No. 18: 112 cows. Entered scheme 30 August 1978

This is a well-managed herd with an enthusiastic owner who has put into practice all the advice given on mastitis control. Originally he was not in the position to replace infected cows by culling, although latterly this has been carried out as heifers came in. He states that his main gain from the scheme was in increased consciousness of mastitis and the control and diagnosis of chronic cases.

Twenty cows were culled because of chronic *S.aureus* infection. Repeated dry-cow therapy had been administered unsuccessfully to several of these cows.

At a loss of R175 per cow (½ value of sound cow) his initial loss was 20 × R175 = R3 500, but some would have been culled soon on grounds of age, etc. Culling was carried out over the latter part of 1979 and early 1980.

Compared with the year of entering the scheme, his average per-cow milk production has increased by 2ℓ/d. At 20c/ℓ this gives an extra turnover of 112 × 20 × 365 = R8 175. In addition the use of antibiotics has decreased by 50 %, a saving of R600.

Loss of milk due to mastitis and after treatment is considerably less and has been estimated now at 30 % of what it was, resulting in a saving of R400. This would be a total saving per annum of R9 175, so that culling losses are easily recovered and will never be as high again with continued participation in the Allerton scheme.

The owner is delighted with the results and will continue indefinitely in the scheme. No extra capital costs were involved in the milking routine which was good.

Six-monthly mean SCC to March 1977: 1 205 000
Six-monthly mean SCC to March 1980: 340 000

Herd No. 67: 107 cows. Entered sheme 21 November 1978

This is a well-managed herd with an enthusiastic husband-and-wife team as owners. Capital was in short supply and cattle were suffering from mastitis, as is usually the case when purchased on open sales. Durban had been critical of SCC for some time and owners were in despair when assistance was requested. Once in the scheme, advice was followed to the letter, resulting in the culling of 30 cows, all chronically infected with *S.aureus*. (Repeated dry-cow therapy appeared to be effective in curing others, but this awaits bacteriological confirmation at the 6-monthly test.)

This loss was estimated at:

Friesland	20 × R300 (½)
Jersey	10 × R150 (½)
Total	R7 500

In any event, several cows would have been routinely culled on grounds of age, etc.

Comparative production figures 12 months after entering scheme at an assessed increase of 2,8 litres per cow per day showed an increased turnover as follows: 107 × 2,8/20 × 365 = R21 870. In addition, the saving on antibiotics was R1 000 and on disposal of milk it was R600, giving a total saving of R22 470. The cost of culling was easily recovered. Home-bred heifers were available for replacement. No capital outlay was required to improve milking procedure.

Six-monthly means SCC to March 1978: 1 173 000
Six-monthly means SCC to March 1980: 864 000

The owners now feel optimistic about future prospects and are enthusiastic supporters of the scheme.

DISCUSSION

The most difficult aspect of mastitis control work is, in many instances, persuading the milk producer that he has a real and costly problem in his herd. Once the owner is convinced that a herd udder health problem exists and that it is causing a substantial preventable loss of income, one is assured of co-operation in any programme to combat the disease. The authors are agreed that being able to provide dairymen with regular SCC of their herd milk is the best known means of making them aware of the extent of the mastitis problem existing in their herds.

It is difficult to convince some dairymen of the benefits to be reaped by reducing the herd SCC and others are impossible to convince. Yet others will be stimulated to act merely on receipt of high SCC on their bulk milk of increased low-lactose incidence. They will introduce proven control measures such as teat dipping and dry-cow therapy, and in many instances where the standard of dairy supervision is good, the results are gratifying.

Control of mastitis is a herd-management problem and it is a foolish waste of time and money to attempt to improve the udder health of a herd run by a person who shows little or no interest in the problem. Certainly the greater the interest shown by the farmer and the better his co-operation with his veterinary advisers, the better will be the end-result.

Another difficulty is weaning the farmer away from the idea that mastitis control is solely a matter of intramammary antibiotic therapy of clinical cases. He has to be shown why he should use the correct antibiotic for clinical cases, and he must be made aware of the value of treating subclinical infections during the dry period, but he must be taught not to rely on these measures alone to solve the herd problem.

The most common deficiencies and faults found in herds experiencing a high SCC have been as follows:

(i) Milking in unhygienic surroundings – parlour or shed, wet and dirty
(ii) Milking-machine operation inefficient – frequently a lack of vacuum reserve, sluggish pulsation, etc.
(iii) Inadequate stimulation of "let down" (especially necessary during first 12 weeks of lactation)
(iv) Poor placement of teat cups and rough cluster removal
(v) Overmilking
(vi) Extended machine "stripping"
(vii) Failure to apply teat-dip immediately cluster is removed
(viii) Failure to diagnose low-grade clinical mastitis cases. Either the operator does not examine the strip-cup or he does not know what to look for or he fails to report the finding of abnormal milk. (In this regard it has been found that only a strip-cup with a black background is of any use – a strip-cup with fine wire gauze is useless.)
(ix) Failure to treat clinical cases with the full course of the correct antibiotics
(x) Milking left to poorly trained staff without constant supervision by some knowledgeable person in authority
(xi) Failure to recognise and cull chronic cases. (It has been found in practice that cows culled in herds where assistance has been called for in controlling mastitis tend to be the older members of the herd, many of which are due for culling in any event. Also, a surprisingly large proportion of these culled on account of mastitis were found to be difficult breeders or affected by footrot)

When most of the above factors are corrected or at least improved and when problem cows have been identified and suitably treated or eliminated, it has been shown that the mastitis picture can be quickly improved in most herds. However, the problem varies from farm to farm, viz. extent of infection, type of infection, availability of replacement heifers, availability of finance, need to supply milk to a quota, etc, so that the advice given on mastitis control has to be adjusted to suit each herd situation.

The majority of farmers in the scheme could easily correct most of these deficiencies quickly when these were pointed out, but the recognition and culling of chronic cases had been the greatest difficulty in the past. If drastic culling is accepted, progress will be certain; delay will only lead to further spread of infection. This applies particularly to *S.aureus* infection. In our work, it was found that *S.aureus* formed 63 % of the known pathogens recovered from routine milk-samples.

The SCC scheme has been extremely popular from the start, indicating that it filled a need and was welcomed by most dairymen. Not all the latter supply fresh milk to Durban, and increasing number of industrial milk producers are entering the scheme. Some 124 herds have been accepted and many more are being surveyed. This involves 13 000 dairy cattle.

A charge of R20 is now made for full membership to the scheme, which in fact recovers only some 20 % of the actual cost to the Government on an average-sized herd. The continuous nature of the scheme is stressed to farmer members and their veterinary advisers. Progress is monitored by a 6-monthly herd SCC check and in addition a bulk-tank check is made monthly on milk supplied to Durban.

Very few farmers have dropped out of the scheme, indicating the value they place on this. Follow-up work on the farm is left strictly to the farmer and his veterinary surgeon, and further visits are not normally made after the initial control visits. It is anticipated that most veterinary practices can incorporate the disciplines of the scheme into their herd-health plans. This has already been the case in Natal. It remains the authors' firm opinion that a logical and workable system of mastitis control gives the veterinary profession an opportunity to grasp which, in many other countries, has been lost to non-veterinarians.

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BACTERIAL CONTAMINATION OF WARM CARCASE SURFACES: THE RELATION OF TOTAL AEROBIC AND COLIFORM COUNTS TO THE RECOVERY OF *ESCHERICHIA COLI* I

B. McCULLOCH* and C.J. WHITEHEAD**

ABSTRACT: McCulloch B.; Whitehead C.J. **Bacterial contamination of warm carcase surfaces: the relation of total aerobic and coliform counts to the recovery of *Escherichia coli I*.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 119–122 (En) Veterinary Laboratory, Box 41, 6140 Grahamstown, Rep. of South Africa.

By an indirect contact method, the total numbers of aerobic and coliform bacteria and of *Escherichia coli* I per cm^2 on the surfaces of warm carcases of 498 cattle, 426 sheep and 499 pigs were established. Total and *E. coli* I counts were classified in geometric progression, the classifications being used to monitor levels of contamination. The highest levels were found on pigs. *E. coli* I was frequently isolated from pig surfaces and only sporadically from sheep and cattle. The recovery of *E. coli* I was related to the overall extent of bacterial contamination. Levels of contamination and the prevalence of *E. coli* I are illustrated by bar-graph arrangements.

INTRODUCTION

In the meat industry, both spoilage and pathogenic bacteria require consideration. Total bacterial growth counts are of use in assessment of keeping quality and in determination of the hygiene of slaughter procedures. Salmonellae are grouped among the most pathogenic bacteria encountered and are responsible for many incidents of food poisoning. Salmonellae are often found in the bowel contents and faeces of apparently normal animals[1257]. Isolation of *Escherichia coli* I from meat is widely regarded as reliable evidence of bowel content spillage and/or faecal pollution; indeed, *E. coli* I is often used as an indicator organism.

This investigation was undertaken to assess whether the total number of aerobic and coliform on the surfaces of the carcases of freshly slaughtered cattle, sheep and pigs could be related to recovery of the *E. coli* I "indicator" organism from such surfaces and to consider ways of their separate and combined expression.

MATERIALS AND METHODS

The surfaces of 498 cattle, 426 sheep and 499 pig carcases were examined bacteriologically at the Port Elizabeth abattoir.

Examination techniques, culture methods and counting procedures

Carcases were examined warm, as they came off the line. Indirect contact examination of a carcase was effected near the left axilla by means of a sterile universal container bottle top as described in an earlier report[6]. Each carcase surface bottle top inoculum was carried from the carcase to plate count agar (Oxoid) and from their directly to MacConkey agar (Oxoid), from where the total and coliform counts, respectively, were determined.

Bottle top contact impressions yielded growth of coliform organisms on MacConkey agar in the case of 17 cattle, 40 sheep and 189 pigs. Several typical coliform colonies were picked off about one third of these positive plates from each species of animal and subjected to procedures aimed at demonstrating the production of gas and indole, respectively, in MacConkey broth purple and tryptone water (Oxford) incubated at 44 °C for 24 h. The percentage of coliforms found to be *E. coli* I was calculated daily for each species. Coliform counts for the relevant day were adjusted accordingly and the *E. coli* I count assessed.

The counting procedure was as previously described[6]; counts were carried out after incubation for 20 h at 37 °C, with the aid of a stereomicroscope and a grid outline. Three indirect colony counts per cm^2 were selected for final assessment:

1. The 4 × Highest Square Count of all colonies (4 × HSC – AC), previously called the 4 × HSC count[6], was determined from plate count agar.
2. The Circle Count of *E. coli* I colonies (CC – *E. coli* I) was assessed from the related MacConkey agar coliform count.
3. The 4 × Highest Square Count of *E. coli* I (4 × HSC – *E. coli* I) was also assessed from the related MacConkey agar coliform count.

Count classifications and statistical analysis

The bacterial counts on cattle, sheep and pig carcases were separately classified and analysed. The overall extent of bacterial contamination was expressed by the 4 × HSC – AC, the extent of *E. coli* I contamination by the CC – *E. coli* I and the 4 × HSC – *E. coli* I. The three colony counts were classified in geometric progression as previously described[6]; first term/common ratio – 4/2.

Unclassified 4 × HSC – AC, CC – *E. coli* I and 4 × HSC – *E. coli* I were subjected to regression analyses. Initial analyses were restricted to data from carcases which yielded *E. coli* I as indicated by the CC – *E. coli* I (Table 1; 7 cattle, 28 sheep and 178 pig carcases). In repeat analyses, data were drawn from all carcases with bacterial contamination reflected by the 4 × HSC – AC (Table 1; 195 cattle, 295 sheep and 471 pig carcases).

Monitoring of bacteriological contamination

Bacterial contamination of cattle, sheep and pig carcase surfaces was monitored on 5 occasions over a 15-month period. Each species was separately assessed.

The overall extent of bacterial contamination was

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Table 1: REGRESSION ANALYSIS STUDIES OF INDIRECT CONTACT, WARM CARCASE, UNCLASSIFIED COLONY COUNTS PER cm²

Counting procedures	Species	Carcase totals	Regression analysis; data related to zero CC – *E. coli* I excluded			Regression analysis; data related to zero 4 × HSC – AC excluded		
			n	r	$\frac{r^*}{S\,E\ of\ r}$	n	r	$\frac{r^*}{S\,E\ of\ r}$
4 × HSC – AC/ CC – *E. coli*	Cattle	498	7	0,6415 insign.	1,5713 insign.	195	0,1143 insign.	1,5920 insign.
4 × HSC – AC/ 4 × HSC – *E. coli* I			7	0,7196 insign.	1,7627 insign.	195	0,1215 insign.	1,6923 insign.
CC – *E. coli* I / 4 × HSC – *E. coli* I			7	0,9468 P<0,01	2,3192 sign.			
4 × HSC – AC/ CC – *E. coli* I	Sheep	426	28	0,0776 insign.	0,4032 insign.	295	0,0947 insign.	1,6238 insign.
4 × HSC – AC/ 4 × HSC – *E. coli* I			28	0,2056 insign.	1,0683 insign.	295	0,1250 P<0,05	2,1433 sign.
CC – *E. coli* I / 4 × HSC – *E. coli* I			28	0,9265 P<0,001	4,8142 sign.			
4 × HSC – AC/ CC – *E. coli* I	Pig	499	178	0,2821 P<0,001	3,7531 sign.	471	0,3142 P<0,001	6,8117 sign.
4 × HSC – AC/ 4 × HSC – *E. coli* I			178	0,2226 P<0,01	2,9615 sign.	471	0,2850 P<0,001	6,1787 sign.
CC – *E. coli* I / 4 × HSC – *E. coli* I			178	0,9207 P<0,001	12,2491 sign.			

*Values of r/S E of r greater than 2 were regarded as evidence of significance. Values of r/S E of r between 2 and -2 were regarded as evidence of insignificance. S E of r = $1/\sqrt{n-1}$

determined; the 4 × HSC – AC were classified. The classification percentage figures were recorded in tabular form (Table 2). The count classification percentage figures for the separate dates of trial were depicted by bar-graph arrangement (Fig. 1).

The presence of *E. coli* I growth was illustrated on the 4 × HSC – AC bar-graph arrangement. The proportions of carcases which showed a 4 × HSC – *E. coli* I presence valued at 4 or more were shaded onto the relevant sections of the bar-graphs (Fig. 1).

The extent of *E. coli* I contamination was noted. Classified 4 × HSC – *E. coli* I were grouped together for the whole period and expressed in percentage form (Table 3).

RESULTS

Statistical analyses (498 cattle, 426 sheep and 499 pig carcases)

Regression analyses of unclassified 4 × HSC – AC, CC – *E. coli* I and 4 × HSC – *E. coli* I gave varying results according to species. In the case of cattle and sheep carcases the respective regression analyses showed insignificant relationships between the 4 × HSC – AC and the CC – *E. coli* I and between the 4 × HSC – AC and the 4 × HSC – *E. coli* I, where data related to zero CC – *E. coli* I were excluded (Table 1; 7 cattle and 28 sheep carcases). Repeat analyses which excluded data related to zero 4 × HSC – AC counts, were in insignificant relationship for cattle (Table 1; 195 cattle carcases). In the repeat analyses of sheep data, insignificant relationship was noted between the 4 × HSC – AC and the CC – *E. coli* I; some evidence of relationship ($P < 0,05$) was noted between the 4 × HSC – AC and the 4 × HSC – *E. coli* I (Table 1; 295 sheep carcases). In pigs there were significant relationships between the 4 × HSC – AC and the CC – *E. coli* I ($P < 0,001$) and between the 4 × HSC – AC and the 4 × HSC – *E. coli* I ($P < 0,01$), where data related to zero CC – *E. coli* I were excluded (Table 1: 178 pig carcases); the repeat analyses also showed significant re-

Table 2: ALL COLONY COUNT CLASSIFICATIONS OVER A 15-MONTH PERIOD. INDIRECT CONTACT, WARM CARCASE COLONY COUNTS PER cm², 4 × HSC – AC.

	Percentage of carcases in each count classification									
Count Classifications	I	II	III	IV	V	VI	VII	VIII	IX	Carcase totals
Counts per cm²	0–3	4–7	8–15	16–31	32–63	64–127	128–255	256–511	512–1023	
Cattle	60,9	13,3	11,8	5,6	3,8	2,8	1,2	0,6		498
Sheep	30,8	20,9	21,4	14,5	8,4	3,5	0,5			426
Pig	5,6	2,2	3,6	7,0	9,2	9,4	19,7	34,5	8,8	499

Table 3: *E. COLI* I COUNT CLASSIFICATIONS OVER A 15-MONTH PERIOD. INDIRECT CONTACT, WARM CARCASE, *E. COLI* I COUNTS PER cm², 4 × HSC – *E. COLI* I.

	Percentage of carcases in each count classification								
Count Classifications	I	II	III	IV	V	VI	VII	VIII	Carcase totals
Colony counts per cm²	0–3	4–7	8–15	16–31	32–63	64–127	128–255	256–511	
Cattle	98,8	0,4	0,6	0,2					498
Sheep	96,7	2,4	0,7	0,2					426
Pig	79,6	9,6	8,0	1,8	0,6	0,4			499

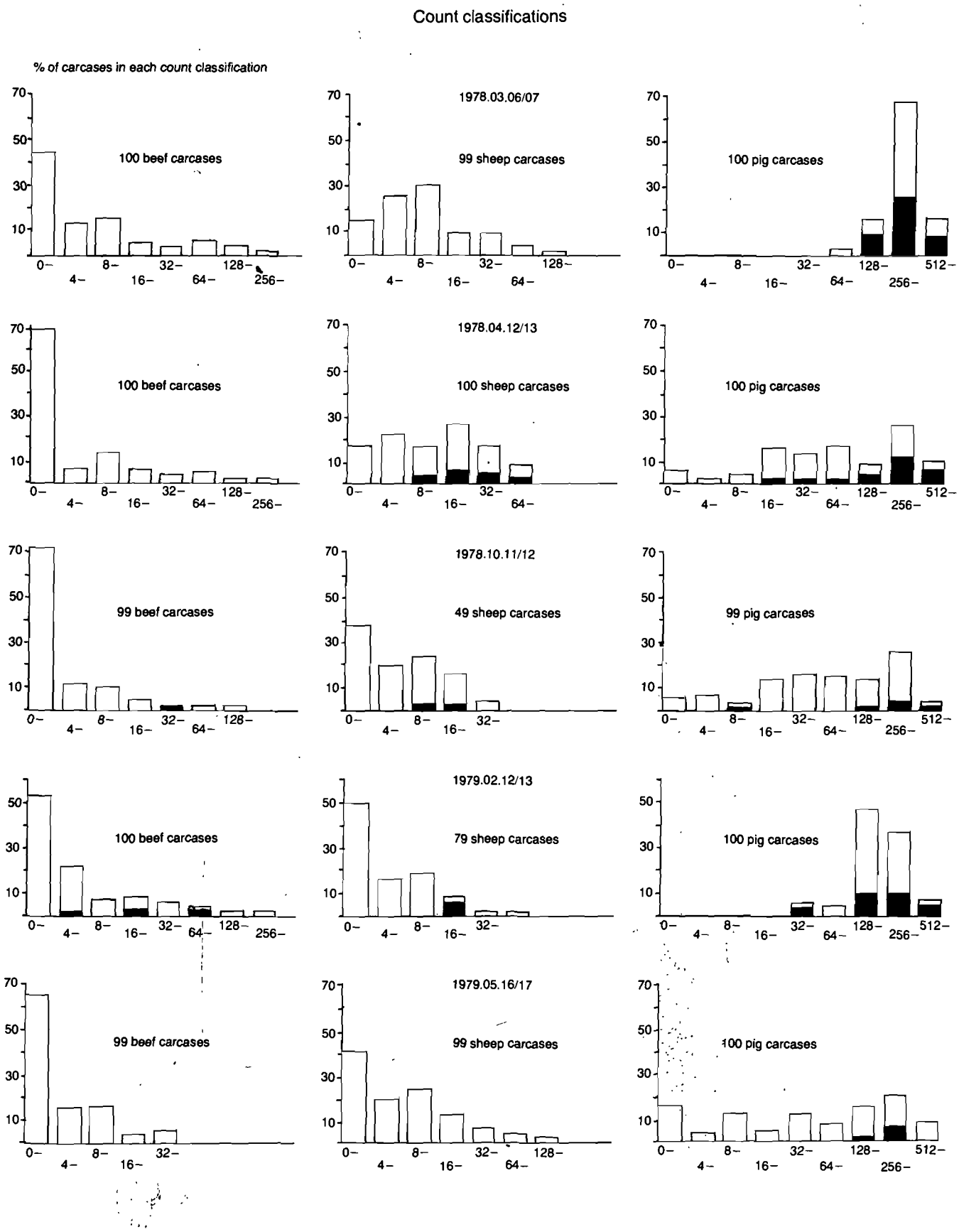

Fig. 1 Beef, sheep and pig carcases: bacteriological monitoring, bar-graph arrangements to illustrate the density of bacterial growth (indirect contact, warm carcases, 4 × HSC – AC count classifications) and *E. coli* I presence (the darkened areas of the bar-graphs representing the proportion of each 4 × HSC – AC count classification percentage with an *E. coli* I presence)

lationships ($P < 0,001$, $P < 0,001$) (Table 1; 471 pig carcases).

In cattle, sheep and pig carcases there were close relationships between the CC – *E. coli* I and the 4 × HSC – *E. coli* I ($P < 0,01$, $P < 0,001$, $P < 0,001$ respectively) (Table 1; 7 cattle, 28 sheep and 178 pig carcases – data related to zero CC – *E. coli* I excluded).

Monitoring of bacterial contamination (498 cattle, 426 sheep and 499 pig carcases)

The overall extent of carcase contamination was expressed by the 4 × HSC – AC. The 4 × HSC – AC classification percentage figures were lower for cattle and sheep than for pigs (Table 2, Fig. 1). By and large only counts from pigs fell into the very high count classification ranges.

E. coli I contamination was not marked on cattle or sheep carcases (Fig. 1). Pig carcases were extensively contaminated; many carcases in high 4 × HSC – AC classifications yielded *E. coli* I (Fig. 1). Likewise, percentage presentation of classified 4 × HSC – *E. coli* I for the whole of the 15-month period of observation illustrated low levels of *E. coli* I contamination on cattle and sheep carcases and higher levels on those of pigs (Table 3).

DISCUSSION

Monitoring of bacterial contamination

The heaviest bacterial contamination was seen on pigs, much lower on sheep and still lower on cattle. In the case of cattle and sheep the isolation of *E. coli* I was sporadic and it was therefore not possible to show close relationships between the 4 × HSC – AC and the CC – *E. coli* I or between the 4 × HSC – AC and the 4 × HSC – *E. coli* I (Table 1). In pigs however, where the degree of contamination was greater, close relationships were established (Table 1). It was not possible to establish whether these close relationships in pigs represented an association between pigs and *E. coli* I counts or an association between overall density of bacterial growth and *E. coli* I counts. It should be borne in mind that both factors were probably involved as the pigs were not skinned.

Recovery of *E. coli* I was expressed as a CC – *E. coli* I and as a 4 × HSC – *E. coli* I. There were close relationships between the CC – *E. coli* I and the 4 × HSC – *E. coli* I (Table 1: cattle, $P < 0,01$; sheep $P < 0,001$; pigs $P < 0,001$). Nevertheless, it was considered, on the basis of simplicity of presentation, that the *E. coli* I prevalence was best illustrated by proportional representation of 4 × HSC – *E. coli* I on the 4 × HSC – AC bar-graph arrangement (Fig. 1).

Bacterial contamination in the meat industry

Many food animals harbour salmonellae. Various stress factors, largely associated with marketing, facilitate cross-infections and stimulate quiescent enteric ones[3,4,7,9,10]. As a result, large numbers of salmonellae may be excreted from the bowel pending slaughter. In this regard contamination of carcase surfaces with bowel contents of faecal material is therefore viewed with concern[8]. *E. coli* I a normal bowel inhabitant, is used to monitor such contamination.

In conditions similar to those in which this work was carried out it would be reasonable to expect that where warm carcase aerobic colony surface counts were high, *E. coli* I would be readily demonstrable. Carcase pollution with material of enteric origin would be the inference. Counts performed on chilled carcases, however, may need separate consideration. *E. coli* I multiplies readily outside the animal body and in all probability fairly readily in the meat distribution environment[8]. Some of the *E. coli* I counts from chilled carcases could be associated with "free-living forms" and in this circumstance misrepresentation of the position could follow.

Because examination of warm carcases took place early in the distribution chain, the results are regarded as more directly related to the slaughtering procedure.

In this investigation, warm carcase contamination patterns were expressed in a practical manner by proportional representation of *E. coli* I recovery on the bar-graph arrangements used to illustrate the overall extent of bacterial contamination.

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A SAFETY TRIAL WITH RAFOXANIDE IN SHEEP

G.E. SWAN and J. SCHRÖDER*

ABSTRACT: Swan G.E; Schröder J. **A safety trial with rafoxanide in sheep.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 123–125 (En) MSD Research Centre, Private Bag X3, 1685 Halfway House, Rep. of South Africa.

Six groups of 6 lambs each were treated orally with rafoxanide at dosages ranging from 0 to 37,5 mg/kg live mass in multiples of 7,5 mg/kg. The lambs were slaughtered 27 to 31 d after treatment for *post mortem* examination. No clinical, ophthalmological or pathological changes attributable to treatment were detected in any of the lambs. Possible differential diagnoses and predisposing factors for rafoxanide toxicity are discussed. It is suggested that plasma-rafoxanide assay be used as a diagnostic tool.

INTRODUCTION

Rafoxanide (3'-chloro-4' (p-chlorophenoxy)-3,5-diiodo-salicylanilide) is registered as a fasciolicide and narrow-spectrum nematocide for sheep and cattle. The recommended dosage of the performed suspension (Ranide: MSD) is 7,5 mg/kg by the oral route. At toxic levels, rafoxanide causes blindness in dogs (3 doses of 100 mg/kg day[3]) and sheep (single dose of 150 mg/kg or more[5]), clinically characterised by mydriasis[11] and ophthalmoscopically by papilloedema[3]. Following reports from the field that Ranide was suspected of causing blindness in sheep[10 11], it was decided to do a controlled experiment in the laboratory. This paper describes the clinical and pathological findings in the experimental sheep.

MATERIALS AND METHODS

Experimental animals: Thirty six Merino x German Merino ram lambs, 3–4 months of age. Lambs were housed in concrete-floored pens, fed on chopped lucerne hay and given fresh bore-hole water *ad lib.* The lambs were identified with numbered ear-tags and randomly allocated to 6 groups of 6 lambs each by means of tables of random numbers.

Pre-treatment observations: On 2 July 1976 a blood sample was collected from every lamb in a heparinized evacuated glass tube (Venoject: Terumo). These samples were subjected to determinations of serum alkaline phosphatase (SAP), total bilirubin (Merckotest: E. Merck), and micro-haematocrit. Spectrophotometric measurements were performed on a Zeiss PM2D spectrophotometer. In addition each lamb was examined clinically and ophthalmoscopically for any abnormalities. On 13 July one lamb from each group was necropsied by the methods described below.

Treatment: On 13 July the 6 groups (with 5 lambs per group) were each treated orally with rafoxanide 2,5 % m/v preformed suspension at one of the following dosages – 0; 7,5; 15; 22,5; 30; and 37,5 mg/kg.

Post-treatment observations: Heparinized blood samples were collected from each lamb immediately prior to, and 6; 13,5; 26,5 h, and 3, 6, 9, 13 and 17 d after treatment. These samples were immediately centrifuged at 8 000 rpm for 30 min, the plasma transferred to clean test tubes and stored in a refrigerator for rafoxanide-assay (see below). Two animals from each group were examined clinically and ophthalmoscopically 1, 2, 3, 4, 5, 6, 7, 9, 10 and 13 d after treatment. The lambs in each group were examined in rotation so that each lamb was seen every second or at least third time.

Plasma rafoxanide assay: The method used was developed by Merck & Co. Inc., Rahway, New Jersey (1968 unpublished information): 2,5 ml of plasma was shaken in a test tube with 5 ml ethanol, placed in an ultrasonic generator bath for 15 min., and the protein precipitated by centrifugation for 15 min. Five ml of 1,2N HCl and 3 ml of spectro grade iso-octane was added to 5 ml of supernatant and the mixture briefly centrifuged. The absorbance of the iso-octane phase was then determined at 337 nm, using iso-octane as a blank.

Autopsy: One lamb from each group was necropsied daily from 27 to 31 d after treatment. The lambs were anaesthetized by an intravenous injection of pentobarbitone sodium (Sagatal V: Maybaker) at a dosages of 30 mg/kg. Each lamb was also injected with a dose of 0,5 mg/kg heparin concurrently with the anaesthetic. When the lamb was properly anaesthetized (absence of corneal tactile reflex), both jugular veins and carotid arteries were dissected from the surrounding tissues. The needle of an infusion tube was then inserted into the one carotid artery in the cranial direction and the other carotid ligated. Both jugular veins were severed as soon as the infusion described below started. Approximately 60 min were allowed after death for the brain to fix *in situ,* after which the carcass was opened for necropsy. Specimens of the following organs were taken and preserved in the same solution used to perfuse the brain: lung, myocardium, thymus, liver, spleen, kidney, adrenal, mesenteric lymph node, superficial cervical lymph node, thyroid, testis, optic chiasma, eye (a portion of the fundus, including the point of entry of the optic tract, was subsequently cut out for sectioning), *brachium pontis* (a slice ca. 2 mm thick was removed from the right arm of the *Pons Varolii* for sectioning) and hippocampus (a slice ca. 2 mm thick was removed from the *corpus* for sectioning). Specimens were embedded in paraffin wax by the usual method, for histopathological examination.

Brain perfusion: In a method based upon that described by Morgan[9], each brain was perfused with 3 ℓ of the fixative solution infused via the carotid artery from a height of 60 cm. The solution consisted of 4 % formaldehyde and 1 % glutaraldehyde in Millonig's phosphate buffer: 2,26 % NaH_2PO_4; 2,54 % HaOH; 40 % glucose described by W S Botha Faculty of Veterinary Science Onderstepoort (personal communication 1976).

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RESULTS

Pre-treatment observations: No abnormalities were detected from haematocrit, SAP, or total bilirubin determination. The mean values were 37,3 (35–39) for haematocrit, 176,4 μg/mℓ (154–206) for alkaline phosphatase and 0,1375 μg/mℓ (0,1–0,3) for total bilirubin.

Post-treatment observations: No clinical or ophthalmological abnormalities attributable to treatment were detected in any of the experimental animals. All lambs except 4 (3 non-treated, and 1 treated at 22,5 mg/kg) displayed mild papilloedema of one or both eyes at one or more of the examinations.

Plasma rafoxanide: The plasma rafoxanide curves for the six treatment groups are presented graphically in Fig. 1. With the exception of the group treated at 15 mg/kg, all groups displayed peak values within 24 h of treatment. In the group treated at 15 mg/kg a peak concentration was measured 48 h after treatment. A peak of about 17 μg/mℓ was detected in blood samples collected from the control group from 13,5 to 48 h after treatment. Plasma rafoxanide values declined (rapidly at first and more gradually later) to values ranging from 15,4 μg/mℓ in the lambs treated at 7,5 mg/kg to 27,6 μg/mℓ in lambs treated at 30 mg/kg, 17 days after treatment.

Histopathology: Most animals from *all* treatment groups displayed varying degrees of "spongiosity" in one or more of the nervous tissue sections. These vacuoles were small, not very well defined, and often occurred in the vacinity of capillaries. No cellular reactions were seen in association with this spongiosity. No lesions attributed to treatment were seen in any of the organs examined.

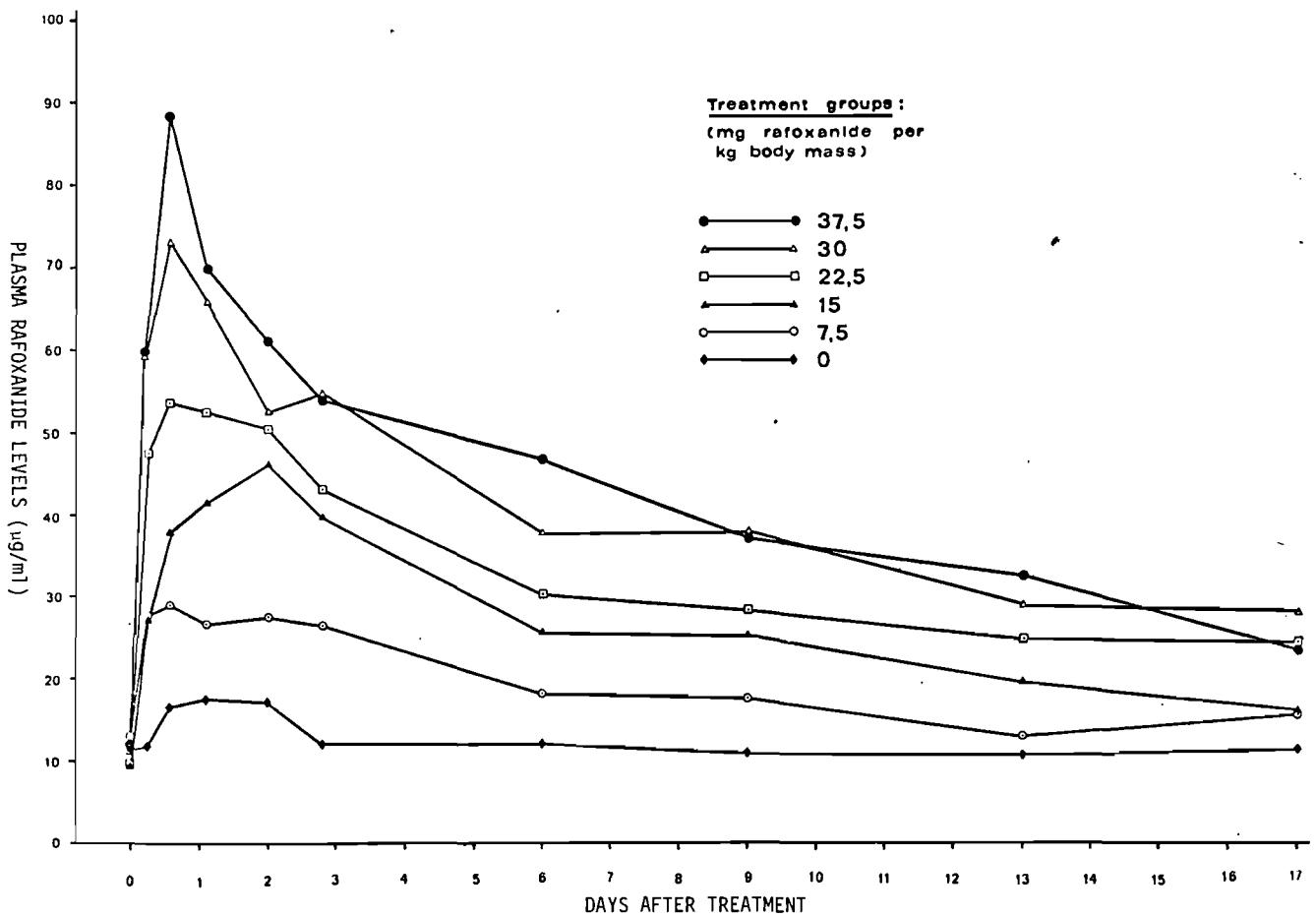

Fig. 1 Mean plasma rafoxanide concentrations (μg/mℓ) in 6 groups of 6 lambs each, 0–17 d after oral treatment.

DISCUSSION

Rafoxanide was introduced to the anthelmintic market in 1970, and by 1975 12 × 10^6 cattle (300 kg live mass) and 289,4 × 10^6 sheep (50 kg live mass) doses had been sold throughout the world in 52 countries without reports of toxicity in sheep at the prescribed dosage (Merck & Co., Inc., Rahway, New Jersay 1976 unpublished information). R W Butler Merck Sharp & Dohme Research Laboratories Ingleburn Australia (unpublished information 1976) treated 46 sheep orally with rafoxanide suspension at 45 mg/kg, with no effect other than transient listlessness and recumbency of 1 of the animals.

In 1976 field reports of sporadic cases of toxicity following the use of Ranide in sheep came to the authors' attention[10,11]. The exact dosage given could not be determined in any of these cases, but it was thought that 37,5 mg/kg (five times the recommended dose) would be sufficient to elicit signs of toxicity under experimental conditions. No signs, ophthalmoscopic abnormalities, gross or microscopic lesions attributable to treatment were seen at any of the dosages employed.

Guilhon et al.[5] indicated that a dose of rafoxanide of 70 mg/kg caused diarrhoea, 150 mg/kg amaurosis and 250 mg/kg prostration. Death occurred from 350 mg/kg, and a sheep treated at 450 mg/kg displayed intense dyspnoea *ante mortem*. They concluded that the dangerous, but not lethal, dose of rafoxanide for

sheep lies in the region of 15 to 20 times the therapeutic dose of 7,5 mg/kg.

The rafoxanide levels ranging from 10,6 to 17,6 $\mu g/m\ell$ in the plasma of untreated lambs were originally thought to be due to some contamination of the cuvette (serial measurement of samples, cuvette drained by suction after each reading). It has since become evident, however, that some reagent background may occur. This can be eliminated by an additional absorbence reading at 328 nm and subtraction of this value from the obtained at 337 nm according to I K Hotson, W J Bliss Merck Sharp & Dohme Research Laboratories Ingleburn Australia (1976 personal communication).

Dogs given 3 to 11 oral doses of 100 mg/kg/d of rafoxanide developed cataracts, papilloedema, vacuolation of the optic tract, optic chiamsa, white matter of the brain and spinal cord and focal vacuolation of the sciatic nerve[3]. The neural and ocular lesions were thought to be caused by an increased cerebrospinal fluid in the cranium, brain oedema and meningitis around the optic tract and optic chiasma.

The "spongiosity" seen in the brains of sheep from all treatment groups is thought to be an artefact of the method of fixation because:

(i) the "lesion" was not a typical *status spongiosus,* the vacuoles being smaller and more irregular in shape; and
(ii) the spongiosity tended to be more severe in the tissue immediately surrounding capillaries.

It is possible that the perfusion was done at too high a pressure. This explanation is not entirely satisfactory, because Morgan[9] perfused the brains of his lambs from a height of 100 cm above the brain and noted the absence of perivascular spaces in control animals.

The mydriasis displayed by field cases[11] closely resembles descriptions of bright blindness (progressive retinal degeneration) caused by ingestion of braken fern *(Pteridium aquilinum)*[12], and of *Helichrysum* toxicity[1] in sheep. The histopathology of the nervous system described for field cases[10 11] is remarkably similar to that described for *Helichrysum* toxicity[1 10]. *Status spongiosus* has also been said to be caused by hexachlorophene[6], isonicotinic acid hydrazine, cuprizone, triethyltin[2 9], hepatic encephalophathy, ammonia toxicity[7], oxyclozanide (Tremerad: Parke Davis & Co.)[8], and chronic copper poisoning[9].

The aforementioned facts suggest that the syndromes ascribed to rafoxanide intoxication have many different causes. Any diagnosis of Ranide toxicity should therefore be made with caution, and preferably confirmed by plasma-rafoxanide assay. The possibility of one or more of the above-mentioned factors predisposing sheep to rafoxanide toxicity may also bear investigation in cases where low doses are incriminated.

Hepatic encephalopathy in cattle is associated with hyperbilirubinaemia, elevated serum alkaline phosphatase levels and decreased excretion of bromosulphthalein[4]. SAP and total bilirubin were determined prior to the onset of the trial to eliminate any sheep suffering from hepatic dysfunction. Apart from a peribiliary infiltration in the livers of some lambs, no biochemical or histological indication of liver pathology was detected.

Most of the reported cases of Ranide-toxicity have occurred in lambs, or sheep in poor condition and on farms with relatively small flocks. Very few South African farmers weigh individual animals prior to anthelmintic (or other mass-dependent) therapy for practical reasons. It is possible that gross overdosage could have occurred in cases of reported toxicity[10 11].

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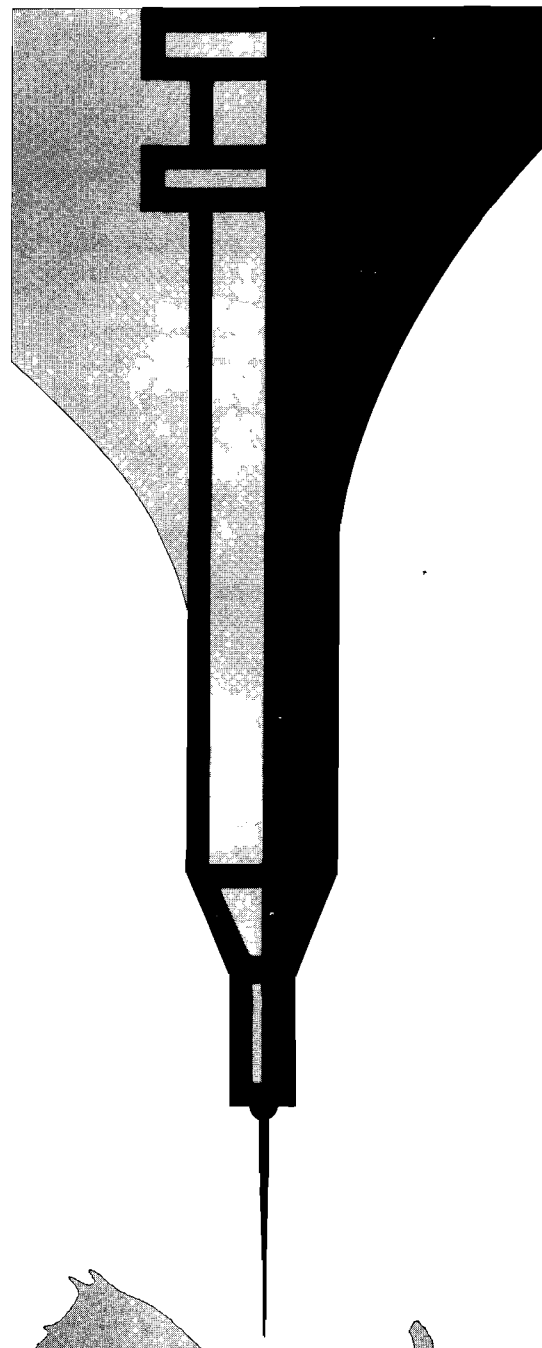

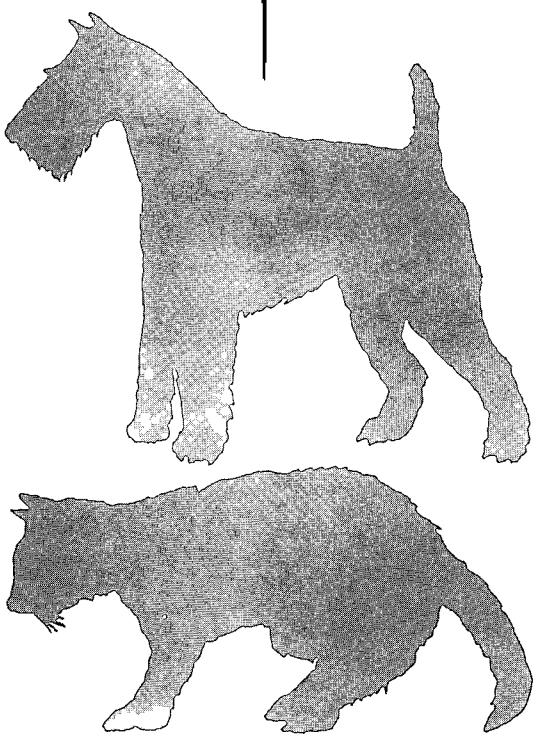

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ANTHELMINTIC EFFICACY OF FENBENDAZOLE PASTE IN EQUINES

F.S. MALAN*, R.K. REINECKE** and R.C. SCIALDO**

ABSTRACT: Malan F.S.; Reinecke R.K.; Scialdo R.C. **Anthelmintic efficacy of fenbendazole paste in equines.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 127–130 (En) Hoechst Research Station, P.O. Box 124, 1320 Malelane, Rep. of South Africa.

A single oral dose of fenbendazole (FBZ) paste at 7,5 mg/kg body mass was given to 5 horses. It was highly effective against adults of the following genera: *Cyathostomum, Cylicostephanus, Cylicondontophorus, Poteriostomum, Cylicocyclus, Triodontophorus, Oesophagodontus* (and other genera belonging to the subfamily Cyathostominae). Similarly, high efficacy was obtained against the adults of the following species: *Oxyuris equi, Strongylus vulgaris, Strongylus equinus* and *Probstmayria vivipara.*

These results were confirmed in 12 horses and in addition FBZ at 7,5 mg/kg was highly effective against *Parascaris equorum, Craterostomum* and *Gyalocephalus.*

INTRODUCTION

The efficacy of fenbendazole (FBZ) (Panacur[R] Hoechst Pharmaceuticals (Pty.) Ltd.) for treatment of adult gastro-intestinal nematode infestations in horses has been demonstrated. At a dosage rate of 5 mg/kg body mass, FBZ is 100 % effective in the removal of adult *Strongylus vulgaris,* 99 % for adult *Strongylus edentatus,* 92 % for Cyathostominae, 100 % for mature *Oxyuris equi* and 80 % for mature *Parascaris equorum*[1].

Treatment of horses with FBZ at a dosage rate of 7,5–60 mg/kg live mass produced negative faecal egg counts for a minimum period of 6 to 8 weeks[6].

This paper reports on critical anthelmintic tests and the faecal worm count in horses.

CRITICAL ANTHELMINTIC TESTS

Hall and Foster[3] developed the critical anthelmintic test. Animals are treated and faeces collected every day until slaughter and the worms expelled counted. At necropsy the surviving worms are counted and efficacy determined as follows:

$$\text{Percentage efficacy} = \frac{\text{Worms expelled}}{\text{Total No. of worms counted}} \times 100$$

MATERIALS AND METHODS

Five horses with positive faecal worm egg counts were housed in separate pens with concrete floors. Hay and water were supplied *ad libitum.* The body mass of each horse was determined before treatment. The 5 horses were orally dosed with FBZ paste at a dosage rate of 7,5 mg/kg body mass.

Every morning and afternoon for 7 days after treatment the total faecal output was collected from each horse. Thereafter the faeces were broken between the fingers and all large worms, i.e. large strongyles, *O.equi* and *P.equorum,* were collected separately. The faeces were thoroughly mixed before being weighed. From this material, 2 aliquots of 1/10 and 1/100 by mass, respectively, were washed through sieves with water (apertures of 150 μm) and the residue on the sieve's surface collected and formalin added. Worms were counted macroscopically in the larger aliquots (1/10) and microscopically in the smaller aliquot (1/100).

Every day 1 horse was slaughtered, beginning 7 days after treatment. The methods used for worm recovery are as follows:

The horse was shot, the throat was cut and the thoracic and abdominal cavities were opened by making an incision along the ventral line of the animal. The left half of the rib cage and the abdominal walls were removed. The thoracic and abdominal organs were removed from the carcass. In order to separate the various sections of the gastro-intestinal tract, double ligatures were tied on either side of the stomach between the small and large intestine. The large intestine was further divided into the caecum, ventral colon and dorsal colon. The contents of the small intestine and stomach were removed, iodine and formalin added and then filtered through a 150 μm sieve.

The total ingesta of the caecum, ventral colon and dorsal colon were each separately mixed in containers and a quarter of the mass of each determined, lugols iodine (20 mℓ per aliquot) was added to kill the worms and formalin (20 mℓ per aliquot) added to fix the worms. The aliquot was then washed through a sieve (150 μm) and the residues on the sieve's surface collected and preserved in 10 % formalin.

The gut wall of each organ was thoroughly washed under a strong stream of running water so that a sample known as wall-washings of each organ was obtained. The wall-washings were processed similarly to the ingesta as described in the above paragraph. From each of the ingesta and wall-washing samples a 1/10 aliquot by volume was made which was examined microscopically. The remaining 9/10 sample was examined macroscopically. Nematodes present were placed in a specimen bottle containing 10 % formalin. Identification of all helminths were made according to Lichtenfels[4].

Digests were prepared from the stomach and a quarter by mass of the small intestine. The mucosa and muscularis layers were removed, placed in 1 liter glass jars and digested by adding 2 % pepsin m/v and 10 N HCl 3 % v/v to 50 mm below the brim. The jars were then placed in a water bath at a temperature of 40 °C for 1,5 h and agitated periodically. The digested mucosae were then sieved (apertures of 38 μm) and the residues on the sieve's surface collected and preserved in 10 % formalin.

The larvae in the mucosa of the caecum, ventral and dorsal colon were counted macroscopically by cutting

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Table 1: HORSES DOSED A SINGLE DOSE OF FBZ PASTE AT 7,5 mg/kg LIVE MASS. SUMMARY OF WORMS RECOVERED FROM FAECES AFTER TREATMENT ANTI-MORTEM AND AT NECROPSY

Horse Number	Age (Years)	Site	EPG	No. of days from treatment to slaughter	*H.muscae*			*H.majus*				*Cyathostominae* A	*Cylicocyclus*	*Cyathostomum*	*Cylicostephanus*	*Poteriostomum*	*Cylicodontophorus*	Total Adult Cyathostominae	*Triodontophorus* L_4	*Triodontophorus* A	*Oesophagodontus robustus*	*Strongylus vulgaris*	*Strongylus edentatus* A	*Strongylus equinus*	*Oxyuris equi*		*Probstmayria vivipara*	Total
					L_4	5th	A	L_4	5th	A	L_4															L_4	A		
1	13	Faeces	1267	+ 7	0	0	0	0	0	0	3225	10762	12306	5995	12463	0	0	41526	0	3	20	36	25	27	957	25	2 778 500	2 824 254
		Necropsy			76	0	289	0	0	407	19546	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	20 967
		Reduction %			0	–	0	–	–	0	14,16	100	100	100	100	–	–	100	–	100	100	100	100	100	100	65,8	100	99,3
2	3	Faeces	1633	+ 8	0	0	0	0	0	0	13309	18264	66135	50281	92301	1621	730	229332	365	9192	5053	48	4	44	3725	130	1 720 000	1 981 202
		Necropsy			956	0	873	0	0	1251	21669	0	0	0	0	0	0	0	0	0	0	0	0	0	682	0	0	26 094
		Reduction %			0	–	0	–	–	0	38,04	100	100	100	100	100	100	100	100	100	100	100	100	100	84,5	100	100	98,7
3	2½	Faeces	533	+ 9	0	0	0	0	0	0	176	296	1518	1406	1076	58	0	4364	0	0	0	0	3	0	0	0	2 000	6 533
		Necropsy			255	679	89	0	0	85	3285	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4 429
		Reduction %			0	0	0	–	–	0	5,09	100	100	100	100	100	–	100	–	–	–	–	100	–	–	–	100	59,6
4	8	Faeces	500	+10	0	0	0	0	0	0	4283	492	18874	3130	8916	0	945	32357	94	4689	94	5	36	70	749	50	0	42 427
		Necropsy			288	728	23	1	207	127	11375	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14 649
		Reduction %			0	0	0	0	0	0	27,4	100	100	100	100	–	100	100	100	100	100	100	100	100	100	100	–	74,3
5	9	Faeces	1333	+11	0	0	0	0	0	0	3002	3249	15830	3299	34142	2081	0	58601	382	4206	107	25	16	151	3866	5	1 519 000	1 589 361
		Necropsy			236	90	0	6	145	24	24058	6	12	67	0	0	0	85	220	24	0	0	4	0	150	0	0	25 222
		Reduction %			0	0	–	0	0	0	11,1	99,8	99,9	98	100	–	–	99,9	63,5	99,4	100	100	80	100	96,3	100	100	98,4

The following stages although reflected in the totals are not included in the table:

Horse 1 — 1 adult *Setaria equina*

Horse 2 — 2 adult *Setaria equina*

Horse 4 — 8 L_4 *Draschia megastoma*
15 5th *Draschia megastoma*
11 L_4 *Strongylus vulgaris*

Horse 5 — 6 L_4 *S.vulgaris*
4 5th *S.vulgaris*
3 L_4 *S.edentatus*
12 Adult *T.axei*
3 Adult *S.westeri*
1 Adult *Schistosoma* sp.

Table 2: WORM EGGS PER GRAM OF FAECES (EPG) AND LARVAL CULTURES FROM THE DAY OF TREATMENT AND WEEKLY FOR NINE WEEKS

Horse's Name	Day of treatment		Weeks after treatment									
			1st – 4th week		5th week		6th week		7th week		9th week	
	e.p.g.	culture	e.p.g.	culture	e.p.g.	culture	e.p.g.	culture	e.p.g.	culture	e.p.g.	culture
Breker	600	+++	0	0	0	0	0	0	0	0	0	0
Brunet	800	++++	0	0	0	0	0	0	0	0	366	+++
Bruno	1133	++++	0	0	0	0	0	0	0	0	0	0
Calvyn	3000	++++	0	0	0	+	33;	+++	666	++++	666	++++
Clara	1400	++++	0	0	0	0	0	0	0	0	0	0
Heidi	867	++++	0	0	0	0	0	0	0	0	0	+
Henna	867	++++	0	0	0	0	0	0	0	0	66	+++
Moshesh	333	++++	0	0	0	0	100	+++	66	+++	66	+++
Peter	1267	++++	0	0	0	0	0	0	0	0	0	0
Radley	1866	++++	0	0	0	0	0	0	0	0	166	++++
Ronel	400	++++	0	0	0	0	0	+	0	+	366	++++
Tini	400	++++	0	0	0	0	0	0	0	+	66	+++

Table 3: FAECAL WORM COUNT OF NEMATODES RECOVERED FROM FAECES AFTER TREATMENT WITH FBZ PASTE (7 5 mg/kg MASS).

Worm species	Horses												Total No. of horses infested
	Ronel	Moshesh	Bruno	Breker	Peter	Radley	Brunet	Tini	Henno	Clara	Heidi	Calvyn	
Habronema muscae	0	100	0	0	0	0	0	0	0	0	0	0	1
Cyathostominae L_4	2700	485	3177	150	1815	1241	23419	233	150	1204	996	1073	12
Cyathostominae A	3923	1552	10159	510	2487	7167	10913	109	730	1276	1344	1175	12
Cylicocyclus	15460	3783	17415	1670	1009	31781	23122	677	1228	257	2338	2748	12
Cyathostomum	4991	2998	7270	260	777	9228	16061	260	638	447	2045	2831	12
Cylicostephanus	6748	2882	13140	0	1754	7201	54192	460	1679	1825	1349	3140	11
Poteriostomum	300	0	3499	0	0	0	6875	157	150	318	0	89	7
Cylicodontophorus	0	0	0	0	0	1332	830	0	121	160	0	0	4
Gyalocephalus	0	0	155	0	0	88	633	0	0	0	0	1698	4
Total Cyathostominae A	31222	11215	51638	2440	6027	56797	112626	1663	4564	4283	7076	11681	
Triodontophorus L_4	128	0	0	0	0	88	0	0	0	0	0	0	2
Triodontophorus A	0	0	0	150	1014	1274	584	280	1153	399	1591	113	9
Craterostomum	0	0	0	0	0	0	2171	0	0	0	0	233	2
S.vulgaris A	10	0	0	0	0	0	2	0	2	1	10	0	5
S.edentatus	0	0	0	0	0	0	0	0	0	0	0	1	1
Oxyuris equi L_4	50	0	487	60	644	0	0	724	433	2114	2637	0	8
Oxyuris equi A	1	0	3	0	20	0	15	60	27	50	3	1	9
Parascaris equorum	0	0	0	2	3	0	0	0	0	4	1	1	5
Total	34311	11800	55305	2802	9523	59400	138817	2960	6329	12314	12314	13103	

the organs into squares of about 400 mm^2 and stretching them out on a firm surface. For confirmation a few larvae were scraped from the mucosa, placed on a glass slide and were examined under a microscope. Digest samples were examined microscopically.

RESULTS

Data are summarized in Tables 1 and 3.

The genera of Cyathostominae are presented in these tables under the heading of the subfamily. Details of the efficacy of FBZ at 7,5 mg/kg against the different genera of Cyathostominae are: *Cylicocyclus* 99,9–100 %, *Cylicodontophorus* 100 %, *Cylicostephanus* 100 %, *Cyathostomum* 98–100 % and *Poteriostomum* 100 %. Against fourth stage larvae (L_4) of Cyathostominae an efficacy of 5,09 to 38 % was obtained.

The incidence of the various genera differed. *Cylicocyclus, Cyathostomum* and *Cylicostephanus* were plentiful while *Cylicodontophorus* was less numerous and present in 2 horses only. The efficacy against other adult Strongylinae varied as follows: *Oesophagodontus robustus* 100 %, *Strongylus vulgaris* 100 %, *Strongylus edentatus* 80–100 %, *Strongylus equinus* 100 % and *Triodontophorus* spp. 99, 4 %.

FBZ efficacy varied against other species as follows: L_4 of *Oxyuris equi* 84,5–100 % and adults 65,8–100 %, *Probstmayria vivipara* 100 %. FBZ was ineffective against *Habronema muscae, Habronema majus, Draschia megastoma,* L_4 and 5th stage of *S.vulgaris* in the anterior mesenteric artery, *Setaria equina, Gastrodiscus* and *Gasterophilus.*

FAECAL WORM COUNT

The term faecal worm count is used to denote a combination of faecal worm egg counts and numbers of worms expelled in the faeces. The host is not killed after treatment.

MATERIALS AND METHODS

Faecal worm egg examination of 12 Boerperd horses were positive for strongyles and 5 of them were infested with *Parascaris equorum*. The mass of each horse was determined and the animals were treated with FBZ paste at a dosage rate of 7,5 mg/kg body mass (Table 2). The horses were individually kept in loose boxes and they were fed sugar cane tops *ad libitum* and water was supplied.

Faeces were collected for a week every morning and afternoon. A 1/10 and 1/100 aliquot by mass of each sample was collected and washed on a 150 μm sieve. The residues were poured into glass jars and formalin was added. The 1/10 samples were examined macroscopically and the 1/100 samples were examined microscopically with a stereoscopic microscope. The larger nematodes i.e. *P.equorum, O.equi* and the large strongyles were removed from the total mass of faeces. Egg counts and faecal cultures were made very week for 9 weeks following treatment.

RESULTS

Results of egg counts and worm recoveries are presented in Tables 2 and 3. The worms were expelled within 5 days of treatment and eggs of both *P.equorum* and strongyles were absent in 11 out of 12 horses for at least 5 weeks after treatment (Table 2).

DISCUSSION

These anthelmintic tests achieved one main objective. Firstly, FBZ paste is more efficient than a powder or liquid because the entire dose is easily administered and readily swallowed, and there is no danger of foreign body pneumonia. Secondly, we reduced the dose of FBZ from 10 mg/kg[5] to 7,5 mg/kg with no loss in efficacy. Thirdly, FBZ was dosed to horses with similar efficacy to that which we reported previously in donkeys.

The critical anthelmintic test is the method of choice for testing efficacy of anthelmintics in horses since more than 95 % of the worms inhabit the caecum and colon[1,2,5,6]. Fifth stage and adult worms from these organs are not digested before they are expelled. This test is unsatisfactory for the stomach worms *H.musca, H.majus* and D.megastoma which were probably digested after they died, and the number expelled in the faeces may not reflect the actual numbers affected by FBZ, nor is it likely that the low efficacy of FBZ ranging from 5 to 38 % against L_4 of Cyathostominae is an accurate reflection of the compound's efficacy. Reinecke et al.[7,8] showed that no reliance could be placed on critical tests against adult abomasal and small intestinal worms nor against larval stages, even if L_4 of *Oesophagostomum columbianum* were present in the caecum and colon of sheep. We feel this could be equally applicable to equines.

Another reason for treating the critical test on L_4 of Cyathostominae in the present report with reserve is the method used for recovery of these larvae from the gut wall. One of us (Malan 1980) counted the worms *in situ* and showed that up to 60 % of L_4 of Cyathostominae were destroyed by pepsin/HCl digestion. This we have subsequently confirmed, hence digestion of the caecal and colonic wall for worm recovery is no longer being practised.

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REGISTRATION OF VETERINARY DRUGS IN AUSTRALIA AND NEW ZEALAND*

I.K. HOTSON**

ABSTRACT: I.K. Hotson; **Registration of Veterinary Drugs in Australia and New Zealand.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 131–134 (En) Merck Sharp & Dohme Research Laboratories, Box 135 Post Office, Ingleburn, 2565, N.S.W., Australia.

In both Australia and New Zealand, the objective of clearance for registration and licensing is to ensure safe and efficient use of chemicals and to recognise the special needs of several different interests, namely 1. end-users concerned with efficacy and possible hazards; 2. the consumers of food products from treated animals; 3. stock owners and vendors who need protection from false claims from competitive products; 4. wildlife and other ecological considerations; 5. protection of overseas markets, with special reference to drug and chemical residues. In Australia the Technical Committee on Veterinary Drugs is responsible for the preliminary clearance of products for mass-medication with respect to efficacy and safety and, in association with the National Health and Medical Research Council Committees, sets residue limits and establishes poison schedules. This committee, however, can only make recommendations to the individual States, who are responsible for administering regulations through relevant State departments. In New Zealand, the Animal Remedies Act (1967) is responsible for the control of manufacture and importation of animal remedies and for the licensing of these for specific purposes. The Animal Remedies Act is administered by an Animal Remedies Board which operates within the New Zealand Ministry of Agriculture and and Fisheries.

Regulation of veterinary drugs is essential to ensure safe and efficient use of these chemicals and to protect several different interests:

The user has to handle a drug very safely and efficiently to avoid any hazards associated with it and to use it in the way and for the purpose for which it was intended.

The consumer of food products from animals that have been treated with the chemical must be protected from danger arising from drug residues.

The vendor should be protected from unsubstantiated or false claims from competitive products.

The environment in which these products are used or stored should be protected, especially as it affects wildlife and beneficial organisms.

The exporter of animal products must be assured that veterinary chemicals will meet requirements of overseas markets with special reference to absence of drug residues.

Australia and New Zealand are both countries that have been heavily dependent on primary industries as a source of export income.

Intensive and extensive livestock production systems have come to rely on veterinary drugs, biologicals and feed additives for the control of parasites, deficiencies and other diseases. There has, therefore, been an awareness of, and a concern for, the efficacy and safety of veterinary drugs. Specific legislation has been in force for about 40 years in these 2 countries.

Although the information required of an applicant for registration of veterinary drugs is similar in both Australia and New Zealand, the legislative responsibility for registration is vested in 6 individual State Governments in Australia, but in a single national Government agency in New Zealand.

REGISTRATION OF VETERINARY DRUGS IN AUSTRALIA

The Australian Constitution gives to the individual sovereign states the responsibility for matters relating to agriculture. The appropriate legislation has been administered by stock medicine boards and has been responsible for regulation of sale of feeds and medicines for livestock.

A need for co-ordination of the activities of individual states, with special regard to difficulties for manufacturers in labelling and registration, matters of public health concern, and the implications for Australian overseas trade, led to creation of a pesticides branch within the Department of Primary Industry in 1967. In 1968 the Technical Sub-committee on Livestock Feed Additives, which was established in 1965 to "make recommendations as to what should be added and at what levels to stock feeds", changed its title to the Technical Committee on Veterinary Drugs (TCVD) and had its terms of reference broadened to include all feed additives and veterinary drugs which might be used in mass medication of food-producing animals[3].

State authorities have agreed that registration of new chemicals in these categories should be withheld at State level until clearance has been obtained from the TCVD.

The TCVD is responsible to the Co-ordinating Committee on Agricultural Chemicals which in turn is responsible to the Standing Committee on Agriculture. The TCVD consists of 1 senior officer representing each State, the Bureau of Animal Health and the National Health and Medical Research Council (NHMRC). The Commonwealth Department of Primary Industry provides a Chairman and the facilities of the Secretariat.

The responsibilities of the Technical Committee on Veterinary Drugs are:

1. To make recommendations as to what should be added and at what levels to stock feeds.
2. To receive submissions and to consider proposals for the use of new feed additives and stock medicines such as dips, sprays, dusts, vaccines and anthelmintics for the mass medication of farm animals, poultry and including medicaments for bees and fish.
3. To evaluate the possible implications of such use in Australia.
4. To evaluate hazards to operators, livestock and consumers of animal products; to recommend precautions in accordance with good husbandry and good

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agricultural practice and to recommend withholding periods appropriate to specific applications.
5. To refer to NHMRC Committees, where appropriate, data for recommendation of:
   1. Poison schedule classification
   2. Maximum residue limits for animal products
6. To make recommendations to state authorities in respect of registration of veterinary drugs.

The individual states carry the legislative responsibility for registration of veterinary drugs for specific uses under specific conditions

An analagous situation exists between the sub-committees of NHMRC and the individual state departments of health.

Thus, clearance from TCVD is required before state authorities will consider for registration any new product intended for use in mass medication of food-producing animals.

At present, the TCVD does not consider clearance for:

1. Remedies for companion animals (dogs, cats, horses)
2. Products with simple change of brand name.
3. Stock feeds other than medicated feeds or premixes.
4. Veterinary ethical drugs which are not agents of mass medication.

Veterinary ethical drugs (available only to, or on prescription from, a veterinarian) are required to be registered in individual states. Clearance by the TCVD is, however, required if any of these are used for mass medication of food animals.

Poison scheduling is a responsibility of state poison legislation. Certain limitations on the sale of veterinary drugs are imposed by poison scheduling by states after recommendation of NHMRC committees. Poison legislation takes precedence over stock medicine legislation.

Imported veterinary biologicals (vaccines, sera, hormones and antibiotics) are controlled under the customs regulations. This control is exercised by the therapeutic substances branch of the Australian Department of Health, which requires compliance with specified quality standards before issuing an import permit. Efficacy and therapeutic safety aspects of biologicals are dealt with by the National Biological Standards Laboratory.

Control over imported materials is also exercised by the Quarantine Act administered in part by the Australian Department of Health. This applies particularly to imported cultures and biologicals and to anything that could harbour bacteria or viruses.

Procedures for clearance of a new chemical

The department of Primary Industry has prepared a protocol of requirements for submissions regarding new veterinary drugs[2].

Submissions should include justification for the use of a particular product and a statement of the claims made for it. Details of product identity including constituents, analytical techniques, formulation, stability, labels and packaging should be provided. Detailed data are required on efficacy, safety, toxicology and residues. Not all research and field trials need to have been carried out in Australia. Safety assessment in laboratory animals, toxicology studies and residue studies can generally be carried out overseas, but there is a specific requirement for local Australian data in support of claims for efficacy and safety in the target animal. Such studies should be carried out in localities typical of the most important fields of use in Australia and preferably where the parasites or diseases occur under their optimal conditions.

Trials showing improvement in mass gain, feed conversion, growth, survival, reproduction or control of parasites and diseases should be replicated and compared against untreated controls using standard methods. Data should be analysed for statistical significance.

Applicants must not withhold or suppress results of trials which do not flatter the product.

In Australia there has been good co-operation between the Technical Committee on Veterinary Drugs, the Australian Veterinary Chemical Association and the Australian Veterinarians in Industry group in providing guidelines for the evaluation of some product groups. These guidelines set out the minimal requirements to be met by applicants in evaluation of compounds such as blowfly specifics, coccidiostats or anthelmintics.

Extensive data are required to demonstrate the safety to target species for which the product is recommended. Data should indicate the margin of safety to such animals and should take into consideration factors such as age, sex, condition, pregnancy, stress, nutritive status and other factors. The product should be tested alone and in combination with other frequently-used products to determine the safety of such combinations. The effect of treatment on reproduction and the effect of repeat treatments should be tested.

The maximum tolerated dose is a useful figure and can provide a basis for calculation of safety index.

$$\text{Safety index} = \frac{\text{Maximum tolerated dose (mg/kg)}}{\text{Recommended dose (mg/kg)}}$$

If the safety index is obviously well in excess of 5, it is not necessary to measure it with precision.

In considering the safety evaluation of a product such as an anthelmintic for mass medication in sheep, the development program usually provides for double therapeutic dose treatment of say 20 000 – 30 000 sheep under all climatic conditions. Animals should be treated in each state, so that effects of nutrition, climate, age, sex, stage of pregnancy, etc., are all evaluated.

Although in countries such as the USA, UK and New Zealand a provisional licence is required for such developmental testing of a new veterinary drug, in Australia there are presently no federal requirements for licensing during a developmental program. However, individual states may require presentation of toxicology and residue data to establish restrictions on such testing before it commences.

Safety to non-target domestic animals should be evaluated so that appropriate warning may be given if adverse effects are detected. This is particularly important in the case of feed additives and medicated feeds which may eaten by animals other than the target species.

The possibility of hazards to wildlife, including beneficial insects, birds, fish and other aquatic creatures, particularly from disposal of unwanted product or used containers, should be evaluated.

The detailed submission on efficacy and safety is sent for evaluation by selected veterinary referees who certify that there is reasonable substantiation of claims made by the applicant. These reports act as a basis for the TCVD consideration of whether or not approval should be given.

Toxicology and Residues

Acute toxicity of a chemical probably gives the most useful rapid indication of potential hazards to domestic animals and to human users. Poison scheduling is therefore based largely on acute oral, dermal and inhalation toxicities.

Ideally, acute oral LD_{50} values should be determined in 3 species, one of which should be a non-rodent. Separate determination should be made on males and females in one test species. It is suggested that rat data should always be given plus data for 2 of dog, cat or monkey.

Sub-acute toxicity determinations may be performed for several purposes.

1. A Maximum Residue Limit (MRL) may be recommended on the basis of 90-day sub-acute feeding trials in rats and dogs where a 2 000-fold safety factor for consumers can be demonstrated.
2. A provisional MRL may sometimes be recommended on the basis of 90-day sub-acute feeding trials in 2 unrelated species, provided that 2-year chronic feeding trials have been commenced.
3. Sub-acute studies are essential as preliminary range finding studies prior to commencement of chronic feeding trials.

Sub-acute studies should demonstrate a "no-effect" level. Groups should include a control group and 3 or more dosage levels, at least one of which should be non-toxic and one toxic. The test material should be given daily by the oral route for the duration of the trial. Observations on growth, behaviour, appetite, feed consumption, clinical abnormalities and mortality should be made, and animals dying during the test should be examined for gross and microscopic abnormalities. Appropriate clinical pathology should be performed and all tumours should be recorded and undergo histopathological examination.

Chronic feeding trials consist of daily oral administration of compound for up to 2 years to 2 species. Usually the rat and the mouse are chosen. Where sub-acute studies reveal that the dog is a more sensitive species, it may be necessary to carry out studies on dogs for 2 years. A "no effect" level should be clearly demonstrated.

Again, a control group and at least 3 dosage levels should be included in chronic feeding trials. Chronic feeding trials are required where a MRL is sought for "finite" residues and in this instance a 100-fold safety factor is required.

Whenever significant levels of residues are involved, carcinogenic, tumourogenic, mutagenic, teratogenic, foetotoxic and neurotoxic evaluation of new compounds should be carried out.

In addition to laboratory animal studies to evaluate safety to humans, teratogenic and foetotoxic effects in the target species should also be investigated. Metabolism, absorption, storage, biochemical change and elimination of active material and any metabolism studies should be carried out in the target species and breakdown of active constituents into metabolites should be determined. These data may enable selection of a particular metabolite as a marker to be used in residue studies.

Arising from toxicity testing, the calculation of the following parameters should be carried out:

1. The "no-effect" level in the most sensitive species tested.
2. Estimate of probable acceptable daily intake for man.
3. Maximum redisue limits.
4. Recommendations regarding MRL for withholding periods.

Results of studies to show residue levels of the original compound and/or metabolites should reflect maximum use level and maximum frequency of use proposed on the product label.

Trials conducted to produce these residue data should employ the formulation intended for marketing in Australia. Radio-labelled trials must be supported by chemical or biological analysis procedures capable of detecting residues. Trials should show the rate of disappearance of residues.

Australian residue data are required for products intended for use to control ectoparasites and also in the case of products for internal use if the use pattern is different from that used overseas.

A veterinary drug subjected to residue study will fall into one of the following categories:

1. No residues occur in tissues or produce (e.g. milk, eggs).
2. Residues do occur but disappear in the course of a reasonably practicable withholding period.
3. Residues do occur and are unavoidable when the product is used in accordance with good husbandry practice. Consumption of food containing these residues will not exceed the acceptable daily human intake for the drug and its metabolites.

Detailed toxicological and residue information is submitted for consideration by referees in the NHMRC in accordance with the requirement of the health authorities. Poison scheduling and MRL and withholding periods are determined by these committees.

In addition to detailed information on efficacy, safety, toxicology and residues, the Technical Committee on Veterinary Drugs also takes into account general information such as registration status of the veterinary drug in Australia and overseas, evidence of approval or rejection by statutory bodies or authorities either in Australia or overseas, and the states of Australia in which application for registration will be made if clearance is approved.

Antibiotics

In the case of clearance of antibiotics for use as a feed additive or in some form of veterinary mass medication, a different procedure may operate. For sale without a veterinarian's prescription it is necessary to obtain exemption from poison schedule 4. The TCVD considers such applications in consultation with NHMRC committees. They require information on the following items:

1. Therapeutic use of the antibiotic, both present and intended, in both human and veterinary medicine.
2. The range of organisms against which *in vitro* or *in vivo* activity has been demonstrated.
3. Available information on the development of resistance.
4. Other antibiotics that may be included in the pattern of resistance.

In Australia, the TCVD and the individual State Stock Medicines Boards do not presently require approval of advertising material in support of claims for new veterinary drugs. However, individual State Stock Medicines Boards can act to prevent misleading or incorrect advertising in relation to specific products.

REGISTRATION OF VETERINARY DRUGS IN NEW ZEALAND

In New Zealand the procedures employed for the registration of veterinary drugs are essentially similar to those in Australia except that in New Zealand a single body, the Animal Remedies Board, is responsible for registration of drugs and for licensing for use.

The Animal Remedies Board has members representing bodies such as the New Zealand Veterinary Association, the Pharmacy Board, the Veterinary Services Council, the Department of Health, analytical chemists, federated farmers, etc. The Registrar is a veterinary surgeon employed in the Animal Health Division of the Ministry of Agriculture and Fisheries.

The Animal Remedies Act, 1967, controls the manufacture, importation, sale and use of remedies used in treating or preventing disease in mammals (other than humans), birds, fishes and captive reptiles.

No product can be manufactured, imported, sold or advertised for sale unless a licence has been first obtained from the Animal Remedies Board.

Six different types of licence may be issued by the Animal Remedies Board and these are:

1. Full ethical licence. This entitles a manufacturer or importer to manufacture or import the remedy and to sell it to a veterinary surgeon or dealer or have it prescribed by a veterinary surgeon.
2. Full non-ethical licence (open seller). This allows the remedy to be placed on the open market without restrictions other than those shown on the label.
3. Full licence with restrictive conditions. A full licence may contain restrictions placed on it by the board. For example, use restricted to certain individuals.
4. Provisional ethical licence. Certain drugs are restricted to use by veterinary surgeons to gain sufficient evidence to enable new drugs to be licensed fully; a provisional licence may be granted while trial work is carried out.
5. Provisional non-ethical licence. New drugs being developed for general use may be approved to allow further information to be gained in support of a full licence.
6. Provisional licence with restrictive conditions. A provisional licence may carry restrictions on the quantity to be manufactured for trial work or some other conditions.

In provisional licenses, a condition applies that results of the work done will be supplied to the Board for evaluation. It is unlikely that a completely new substance would be granted a full licence without 1 or 2 preliminary seasons of provisional licensing. Developmental work in New Zealand is expected before granting of a full licence. Provisional licences are granted for 2-year term, but this may be extended for a further period if the Board believes more time is necessary to obtain more information.

Where necessary, the Animal Remedies Board consults with the Poisons Committee under the Poisons Act to determine poison scheduling before a licence is issued.

The Animal Remedies Board also carries responsibility for approval of labelling of animal remedies and also for approval of any advertising material concerning animal remedies.

The Animal Remedies Board in New Zealand has issued a booklet setting out requirements for licensing of animal remedies[1]. Appendices contain directions regarding the requirements for submissions for licensing and in the case of licensing of new animal remedies, the information required is very similar to that required by the Australian TCVD and State Stock Medicines Boards.

Escalating costs of developing new veterinary drugs have occurred mainly because of increased safety assessment requirements. There is hope for more uniformity internationally in regulatory requirements and the acceptance of studies from many sources, provided that strict controls and good laboratory practices are maintained. It is reasonable to expect acceptance of laboratory animal data for toxicology and residue information, but it is also reasonable to expect a requirement for locally-generated efficacy and safety data in target animals where diseases, parasites, husbandry, etc., are unique to individual countries.

Where good co-operation exists between regulatory authorities, academia and industry, mutually acceptable criteria for regulation have developed and control of the use of veterinary drugs is carried out effectively.

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ASSESSMENT OF RADIOGRAPHIC POSITIONING FOR THE DIAGNOSIS OF NAVICULAR DISEASE IN THE HORSE*

R.J. ROSE**

ABSTRACT: Rose, R.J. **Assessment of radiographic positioning for the diagnosis of navicular disease in the horse.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 135–138 (En) Department of Veterinary Clinical Studies, University of Sydney, N.S.W., 2006, Australia.

Three of the standard radiographic views of the navicular bone were assessed in normal horses and horses with navicular disease to determine the most effective radiographic positioning. Using the upright pedal view, a pastern angulation of 20° from the vertical produced the optimum result when radiographs were taken using a grid. The best result using the high coronary view was obtained with an anode-film distance of 1 000 mm, and a tube-head angle of 50° from the vertical. When the special navicular view described by Morgan was examined, the most satisfactory projection was obtained using an anode-film distance of 900 mm, a tube-head angle of 55° and the primary beam centred between the bulbs of the heel. For the diagnosis of navicular disease, a combination of the upright pedal view and the special navicular view appears most effective.

INTRODUCTION

Since the latter part of the nineteenth century, when navicular disease (ND) was first recognised as a cause of lameness in the horse, there has been much controversy concerning its diagnosis. When Oxspring[5] described a radiographic technique to enable visualization of the navicular bone, radiography appeared to be the definitive diagnostic aid. Since then, variations in radiographic positioning have been suggested[2,4,6,7], but confusion still exists concerning the significance of radiological changes. Adams[1] has stated that radiological changes in the navicular bone are found in less than half the cases of ND, whereas Rose, Taylor and Steel[6] reported significant radiological changes in the navicular bones of all horses when 70 horses with ND were examined. Colles (personal communication) is of the opinion that in many cases of ND, poor radiographic technique is responsible for the apparent normality of the navicular bone.

This study was performed to attempt to define the best radiographic techniques and positioning of the foot that would permit consistent visualization and interpretation of radiographic changes involving the navicular bone.

MATERIALS AND METHODS

A total of five horses was used to evaluate three radiographic positions commonly used to study the navicular bone. Three of the horses had clinical evidence of ND and two had no history of lameness. Agfa Curix 2 film was used for all radiographs.

Upright Pedal View (Fig. 1)

This view is perhaps the most commonly used to visualise the navicular bone and was originally described by Oxspring[5]. To determine the most suitable foot position, a goniometer was used to measure different angles of the pastern when a line was dropped vertically from the anterior angle of the fetlock joint (Fig. 2) and radiographs using par speed screens were taken in these positions. To determine the efficacy of using a grid to improve radiographic detail of the navicular bone, the radiographs of 10 horses diagnosed as having ND were compared with radiographs taken of 10 other horses with ND radiographed using a grid and high-speed screens.

High Coronary View (Fig. 3)

This view is popular in North America and is performed with the horse standing on the x-ray plate. The tube head of the x-ray machine is then angled to centre the beam above the coronary band. Various angles of the tube head and anode-film distances were used to determine the most suitable projection of the navicular bone, using par speed screens.

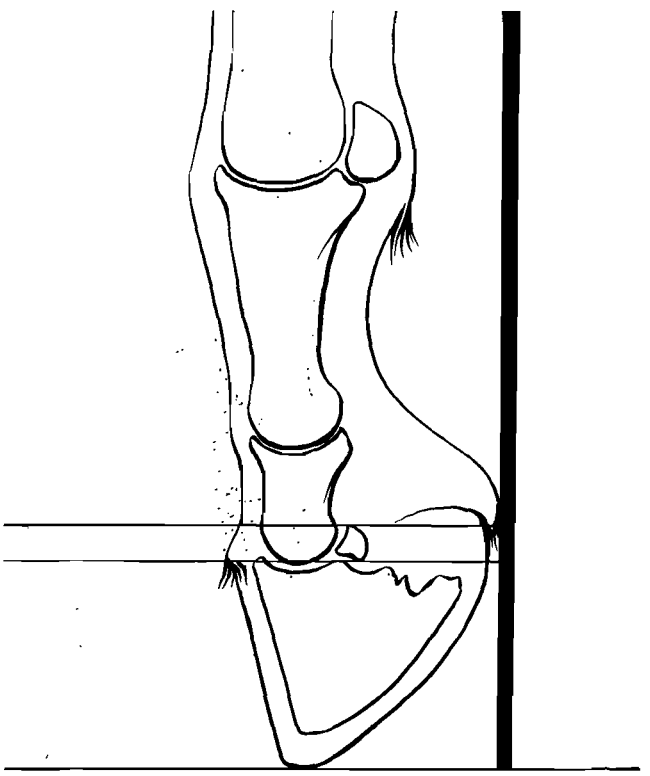

Fig. 1. Radiographic positioning for the upright pedal view.

Special Navicular View (Fig. 4)

This view of the navicular bone was developed by Morgan[4] and involves positioning the x-ray machine just in front of the horse's hind leg. The tube head is angled so that the primary beam is centred between the

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Fig. 2. Goniometer being used to measure angle of the pastern in the assessment of the upright pedal view.

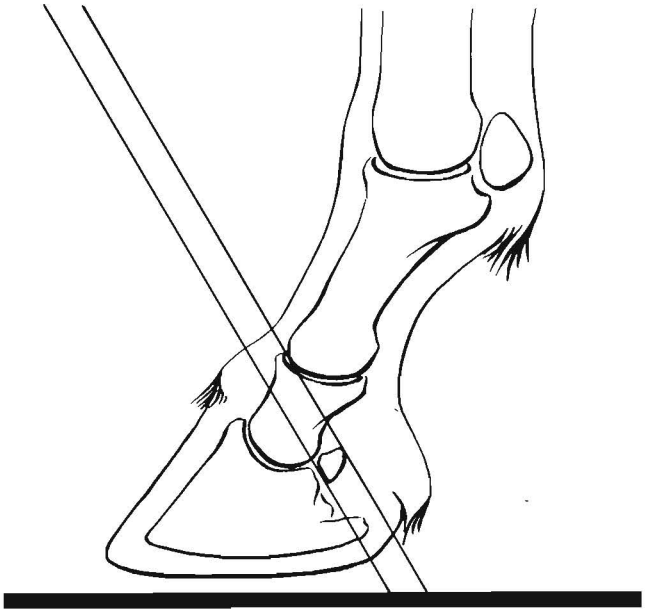

Fig. 3. Radiographic positioning for the high coronary view. Note that a special reinforced cassette should be used.

bulbs of the heels and the horse stands on the cassette. The advantage of the view is that the navicular bone can be seen without it overlying the second phalanx. Various angles of the tube head and anode-film distance were used to determine the best view of the navicular bone, when par speed screens were used.

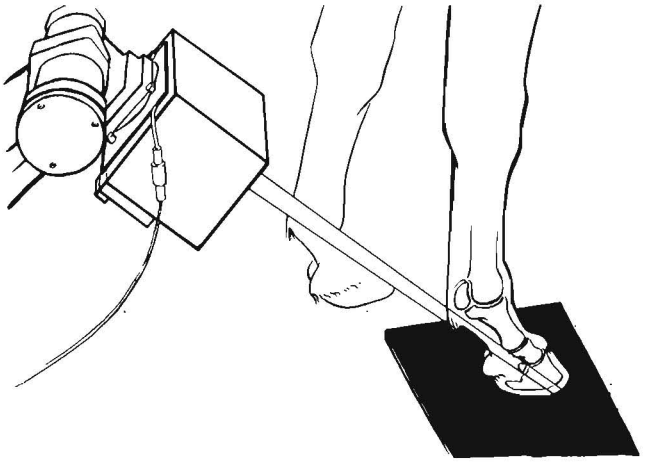

Fig. 4. Radiographic positioning for the special navicular view. Note that a special reinforced cassette should be used.

RESULTS

Upright Pedal View

The most satisfactory view of the navicular bone in both normal horses and those with ND was obtained when a pastern angle of 20° was used and an anode-film distance of 1 000 mm. When the pastern was less angled, the ventral border of the navicular bone came to overlie the coffin joint, resulting in loss of detail. Increasing the pastern angle resulted in distortion of the navicular bone, which became displaced and appeared to attain a more dorsal position over the second phalanx.

The use of a grid resulted in much better detail of the navicular bone in all horses radiographed, when compared to radiographs taken without a grid. This detail could also be improved by coning the primary beam of the x-ray machine so that it was concentrated on the navicular bone. In this situation the primary beam was centred on the anterior midline of the second phalanx, 20 mm above the coronary band. Tracings taken from radiographs of a normal horse and a horse with navicular disease are shown in Figs. 5 and 6.

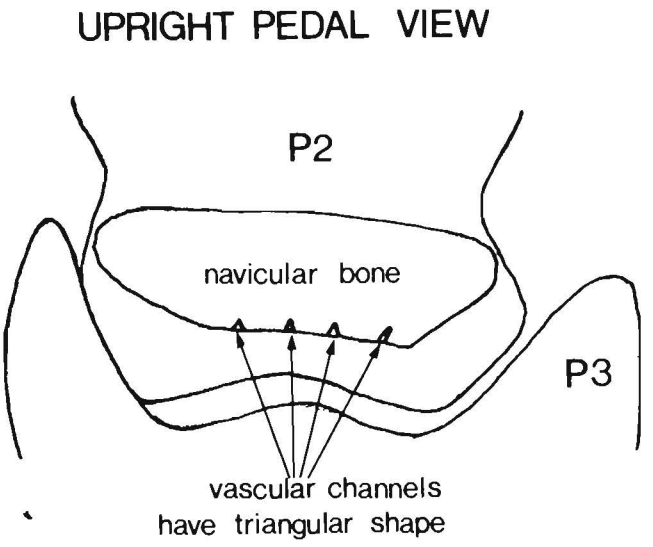

Fig. 5. Tracing of a radiograph for a normal horse, using the upright pedal view.

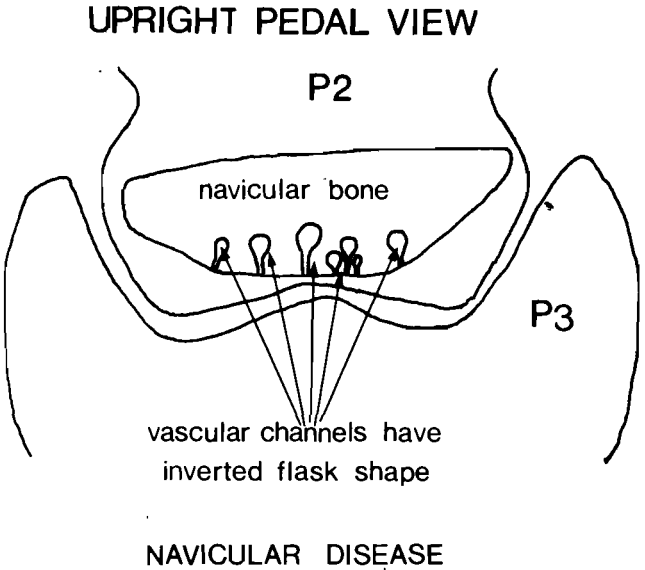

Fig. 6. Tracing of a radiograph of a horse with navicular disease, using the upright pedal view.

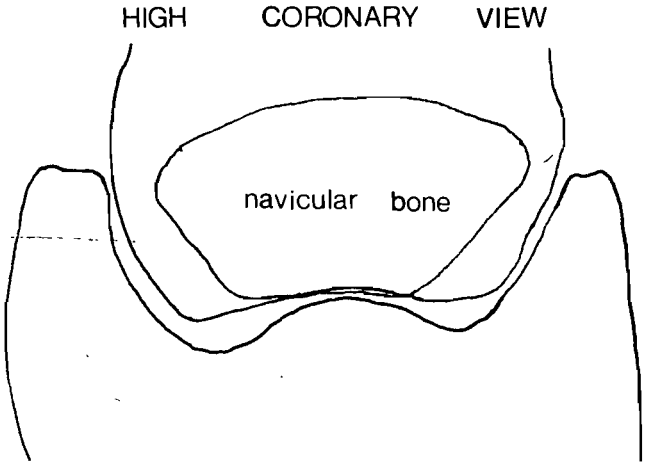

Fig. 7. Tracing of a radiograph taken using the high coronary view.

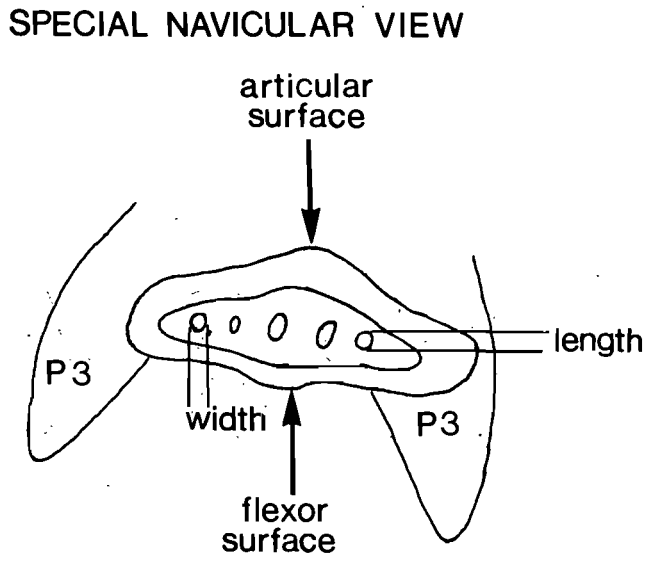

Fig. 8. Tracing of a radiograph taken using the special navicular view.

High Coronary View

The best view of the navicular bone was obtained with a tube head angle of 50° from the vertical and an anode-film distance of 1 000 mm. However, this view resulted in a great deal of magnification and distortion of the navicular bone. As a grid cannot be used with this technique, the detail of the navicular bone is much less than that seen when using the upright pedal view (Fig. 7).

Special Navicular View

Angulation of the tube to 55° from the vertical and an anode-film distance of 900 mm produced the best view of the navicular bone, using this technique. It was found to be important to have the foot being radiographed slightly anterior to the other front leg and to make sure that the direction of the tube head was exactly the same as the longitudinal axis of the foot and pastern. This view permitted excellent visualizaion of the posterior border of the navicular bone and assessment of the size of the vascular channels. It was shown previously that the horses with ND had significantly more vascular channels that were wider and longer than those of normal horses[6]. A tracing of a radiograph showing this view, together with how the vascular channels were measured, is presented in Fig. 8.

DISCUSSION

It is important to standardize radiographic techniques for assessment of changes occurring in the navicular bone. Campbell and Lee[2] reported on positioning for radiography of the navicular bone, but this was in post-mortem specimens of legs, which may not represent the clinical situation. The upright pedal view appears to provide the best assessment of the ventral and dorsal borders of the navicular bone. Increased detail of the vascular channels can be seen when a grid is used in addition to the standard cassette. To accomplish this, longer exposure times are necessary and therefore smaller portable x-ray machines are unsuitable. Most protable machines have a maximum of 30–50 mA available and, therefore, exposure times approaching 1 second are necessary when a grid is used. Colles[3] has shown that there is a change in shape of the vascular channels in horses with navicular disease from a triangular shape (normal) to an inverted flask shape. These changes can be difficult to see without the use of a grid, and therefore small portable x-ray machines are inappropriate if diagnostic radiographs of the navicular bone are required. The use of the high coronary view, although easier to perform than the upright pedal view, results in magnification and distortion of the navicular bone.

The special navicular view can provide additional information to that obtained by the standard radiographic views. In particular, assessment of the flexor surface of the bone is possible, together with good detail of the medullary cavity and size of the vascular channels. The use of the special navicular view can also allow diagnosis of some fractures of the navicular bone, which may not be visible using the standard view.

If a lateral view of the navicular bone is to be used, it is important to have the primary beam centred on the longitudinal axis of the bone. In most cases the lateral view adds little information about the navicular bone, but can permit evaluation of the third phalanx.

It is important, if good detail of the navicular bone is to be achieved, to have a uniform consistency of the

sole. This is obtained by making sure the sulci of the frog are properly cleaned out and packed with a material such as Play-Doh, which will prevent the radiolucent lines that will otherwise appear across the navicular bone.

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ABSTRACT: Belonje, P.C. & Van Den Berg, A., 1980. The use of faecal analyses to estimate the phosphorus intake by grazing sheep. I. The use of pool instead of individual samples. *Onderstepoort Journal of Veterinary Research,* 47, 163–167 (1980).

Analyses of rectal faeces of sheep for phosphorus, calcium and magnesium may be useful to indicate the mineral status of the herbage being consumed. A number of sheep should be used for each pasture, but this would entail a large number of individual analyses. In this experiment it was shown that a pool analysis of faeces did not differ significantly ($P<0,01$) from the arithmetic mean of the individual samples (20). In 13 replications this was true for phosphorus, calcium and magnesium, whether the sheep were non-pregnant, non-lactating, pregnant, or lactating. From the results it was estimated that 3 faecal pellets from at least 30 sheep should be used to make the pool.

Blood was also taken on 11 occasions for pool and individual (20) analyses. No significant difference was found for plasma inorganic phophate, magnesium and total protein, but on 3 occasions there was a significant difference ($P<0,01$) for plasma calcium.

ABSTRACT: Van der Walt, J. G., Procos, J. & Labuschagne, F. J., 1980. Glucose turnover, tolerance and insulin response in wethers, ewes and pregnant ewes in the fed and fasted state. *Onderstepoort Journal of Veterinary Research,* 47, 173–178 (1980).

Glucose turnover parameters were obtained in fed and fasted wethers, ewes and pregnant ewes in their 2nd and 3rd trimesters, using a jugular bolus injection of D-glucose-2-^{3}H. Fasting significantly ($P<0,05$) reduced glucose turnover (*c* 40%) in both the wether and the non-pregnant ewe. A somewhat larger difference (*c* 54%) between the fed and fasted ewes was found in their 3rd trimester of pregnancy due to an increase when fed (*c* 29% higher turnover than in the non-pregnant ewe) rather than a decrease when fasted, since there was no statistical difference ($P<0,1$) between glucose turnover values of pregnant or non-pregnant fasted ewes. Glucose tolerance was estimated from an intrajugular glucose load (a g/kg^{0,75} body mass) in these 3 groups of sheep under both fed and fasted conditions, and the resulting insulin response was followed for 4 h after the injection. Fasting reduced the plasma clearance rate of glucose by *c* 63% in both the wether and the non-pregnant ewe while the reduction was somewhat smaller (*c* 51%) during the 2nd trimester of pregnancy. Only the pregnant ewe group showed a corresponding reduction in the resulting insulin response of 46% which was similar in magnitude to the diminished clearance, indicating that factors other than insulin are responsible for the reduced glucose clearance associated with fasting in the whether and non-pregnant ewe. Despite similar baseline plasma glucose values the glucose load appeared to distribute in a space that was significantly less than that found in all 3 groups of fed sheep when trace amounts were injected.

ABSTRACT: Terblanche, H.M. & Labuschagne, J.M., 1980. Plasma progesterone in cattle. I. Development and validity of the assay. *Onderstepoort Journal of Veterinary Research,* 47, 179–185 (1980).

The development of a practical competitive protein-binding assay for plasma progesterone in cattle is described. With an intra-assay coefficient of variation of 5,46% and an interassay coefficient of variation of 14,25%, the method is sufficiently accurate and sensitive for practical purposes, and for use in routines and surveys. The statistical level of sensitivity was found to be in the region of 0,25 ng/mℓ based on the confidence limits of zero dose and 0,25 ng/mℓ, with the practical sensitivity level at 0,50 ng/mℓ. Method and reagent blanks were found to be negligible. The specificity of the assay is based entirely on the partial specificity of the petroleum ether used for the extraction of progesterone (87,5% extraction, n=141).

ABSTRACT: Van Wyk, J.A. & Gerber, H.M., 1980. A field strain of *Haemonchus contortus* showing slight resistance to rafoxanide. *Onderstepoort Journal of Veterinary Research,* 47, 137–142 (1980).

A field strain of *H. contortus,* already resistant to benzimidazole anthelmintics, was also found to be slightly resistant to rafoxanide. This is apparently the first report of resistance to rafoxanide in a field strain of *H. contortus.*

APPLICATION OF SEVERAL PHYSICAL TECHNIQUES IN THE TOTAL ANALYSIS OF A CANINE URINARY CALCULUS

A.L. RODGERS*, M. MEZZABOTTA**, K.J. MULDER*** and L.R. NASSIMBENI***

ABSTRACT: Rodgers A.L.; Mezzebotta M.; Mulder K.J.; Nassimbeni L.R. **Application of several physical techniques in the total analysis of a canine urinary calculus.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 139–142 (En) Department of Medical Biochemistry, Medical School, University of Cape Town, Observatory, 7925 Cape Town.

A single calculus from the bladder of a Beagle bitch has been analyzed by a multiple technique approach employing x-ray diffraction, infrared spectroscopy, scanning electron microscopy, x-ray fluorescence spectrometry, atomic absorption spectrophotometry and density gradient fractionation. The qualitative and quantitative data obtained showed excellent agreement, lending confidence to such an approach for the evaluation and understanding of stone disease.

INTRODUCTION

Identification of the chemical constituents of animal urinary calculi is of importance in the diagnosis and management of the disease, as different constituents have different aetiologies and require different modes of treatment and prophylaxis. Early studies of the composition of animal stones were limited to chemical analysis in which no distinction between the various calcium phosphates or constituent hydrates could be made[1]. X-ray powder diffraction techniques have been successfully applied in determining the crystalline composition of calculi from deer, horses and dogs[2], one such study involving over 1 000 urinary concretions[3]. More recently, x-ray diffraction techniques were used in a study of the composition of 45 urinary calculi from 8 families of animals[4]. Today, x-ray diffraction is widely used for the analysis of human urinary calculi because it can identify the various crystalline constituents present within a calculus with a certainty that cannot be matched by chemical methods. However, there are certain well-known limitations associated with this technique, the most critical of which is the failure to detect constituents present in low concentrations[5]. Since the minor component may be the primary initiating material of the calculus, with other components having been deposited in secondary processes, identification of *all* the constituents present is vital for the evaluation of the disease. Furthermore, *quantitative* measurements of the relative concentrations of all constituents may provide insight into understanding the physico-chemical processes governing nucleation, growth and aggregation mechanisms. Such data can be obtained by utilization of a mulitple technique approach in which the effects of inherent limitations and shortcomings of any particular procedure are minimized by the inherent advantages of others.

In the present study a calculus from the bladder of an 8-year-old Beagle bitch was analyzed by x-ray powder diffraction, infrared spectroscopy, scanning electron microscopy (plus x-ray energy dispersion), x-ray fluorescence spectrometry, density gradient fractionation and atomic absorption spectrophotometry. This paper describes the application of these techniques and the results obtained and attempts to establish guidelines for a powerful analytical approach to obtaining an accurate picture of stone composition and structure.

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MATERIALS AND METHODS

An 8-year-old Beagle bitch was presented with a history of cystitis with symptoms of haematuria and dysuria. Palpation of the bladder evoked pain. The patient was too fat and tense to permit satisfactory manipulation of the bladder, so a general anaesthetic was administered and radiology revealed a single large calculus in the bladder. The stone (Fig. 1) was removed surgically and the patient made an uneventful recovery.

X-ray diffraction

The calculus was cut using a low speed cutting saw, and samples were obtained from the centre, interior and outer regions of the exposed surface. These were ground to a fine powder and diffraction patterns were recorded on KODAK NS-392 T film by the Debye-Scherrer method using a Philips powder camera of radius 28,65 mm and CuK_α radiation of wavelength 1,5418 Å. The constituents giving rise to the reflections were identified by comparison with the published reference standards of Sutor and Scheidt[6].

X-ray fluorescence

A sample of the stone was prepared by drying at 100 °C and ashing at 1 000 °C. After addition of a flux and melting at 930 °C, a fusion disc of diameter 30 mm was prepared and analyzed for major elements in a computer controlled Siemens SRS 1 automatic x-ray fluorescence spectrometer. International rock standards were used for calibration.

Scanning electron microscopy

After cleaving, a sample of the stone was mounted on an aluminium stub and coated with approximately 500 Å of carbon at 10^{-6} torr in a Balzer's vacuum coater. The specimen was studied using a Cambridge S180 Scanning Electron Microscope operating in the secondary electron collection mode at a normal beam potential of 20 kV and beam current of 500 μA. The microscope was equipped with an energy dispersive x-ray analyzer system which was used for the qualitative determination of Mg, Ca and P.

Density gradient analysis[7]

Initially a column of carbon tetrachloride (ρ = 1,60) and methylene iodide (ρ = 3,32 kg/dm^3) was constructed, as this covered the entire density range of

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commonly occurring urinary calculus constituents. Subsequently, a column of carbon tetrachloride and tetrabromoethane (ρ = 2,96 kg/dm^3) was used for more sensitive determinations. The columns were calibrated by allowing immiscible solutions or crystals of known density to descend through the gradient until their equilibrium positions were reached. A small quantity of dried stone sample was ground to a fine powder and introduced into the column. By reference to the calibration curve, the sample density was obtained and the percentage composition calculated.

Infrared spectroscopy

10 mg of sample, or standard, were added to 90 mg of KBr and placed in a 12 mm diameter Perkin Elmer die in which a disc was pressed at 10 000 kg pressure. The disc was placed in a holder, and spectra were recorded on a Perkin Elmer 180 grating infrared spectrophotometer. All samples were scanned from 4 000 cm^{-1} to 250 cm^{-1}. Analar grade samples of uric acid, hydroxyapatite, struvite, whewhellite, sodium urate and ammonium urate were used as standards.

Atomic Absorption Spectrophotometry

All analyses were carried out on a Varian Techtron Model 1000 atomic absorption spectrophotometer using an air acetylene burner and a calcium/magnesium hollow cathode lamp. Standard solutions were prepared using analar grade reagents and B-grade glassware. The samples and standards were dried under vacuum for 24 hours.

RESULTS

The qualitative and quantitative data obtained are presented in Table 1. The x-ray powder diffraction patterns of the 3 samples all showed the presence of struvite ($MgNH_4PO_4.6H_20$) and hydroxyapatite ($Ca_{19}(PO_4)_6(OH)_2$) (Fig. 2). These 2 components were also identified from the infrared spectrum (Fig. 3). Scanning electron microscopy revealed the presence of large struvite crystals interspersed with or surrounded by small densely packed deposits of hydroxyapatite (Fig. 4 and 5). Energy dispersive x-ray analysis confirmed the presence of Mg and P in the large crystals and Ca and P in the smaller deposits (Fig. 6). In addition x-ray elemental distribution images were recorded for the area shown in Fig. 5 (Fig. 7–9).

Table 1: CHEMICAL COMPOSITION OF THE CALCULUS AS DETERMINED BY THE DIFFERENT TECHNIQUES

Qualitative			Quantitative (wt %)			
XRD	IR	SEM		XRF	Density	AA
STR	STR	STR	STR:	90,5	93,8	91,6
HA	HA	HA	HA:	9,5	6,2	8,4

XRD: x-ray diffraction; IR: infra red; SEM: scanning electron microscopy; XRF: x-ray fluorescence; Density: density column; AA: atomic absorption; STR: struvite; HA: Hydroxyapatite.

Quantative data were obtained using the remaining 3 techniques. X-ray fluorescence spectrometry yielded values for the percentage of Mg, Ca, P and H_2O and these were used in simple stoichiometric calculations to give the relative mass of each component present. In the density column the powdered stone sample did not separate into 2 discrete bands but settled at a column height corresponding to a density of 1,80 kg/dm^3. On the basis of the qualitative data obtained from the x-ray diffraction, infrared and scanning electron microscopy studies, struvite and hydroxyapatite were assumed to be the only components present, thereby permitting calculation of the relative amounts of each from the measured density of the mixture. The densities of the pure components were taken as 1,71 (struvite) and 3,15 kg/dm^3 (hydroxyapatite)[3]. For the atomic absorption analyses, various interference effects were investigated. These showed that while Ca absorbence is unaffected by struvite, Mg absorbence is enhanced by the presence of Ca. Consequently, a "standard additions" method of

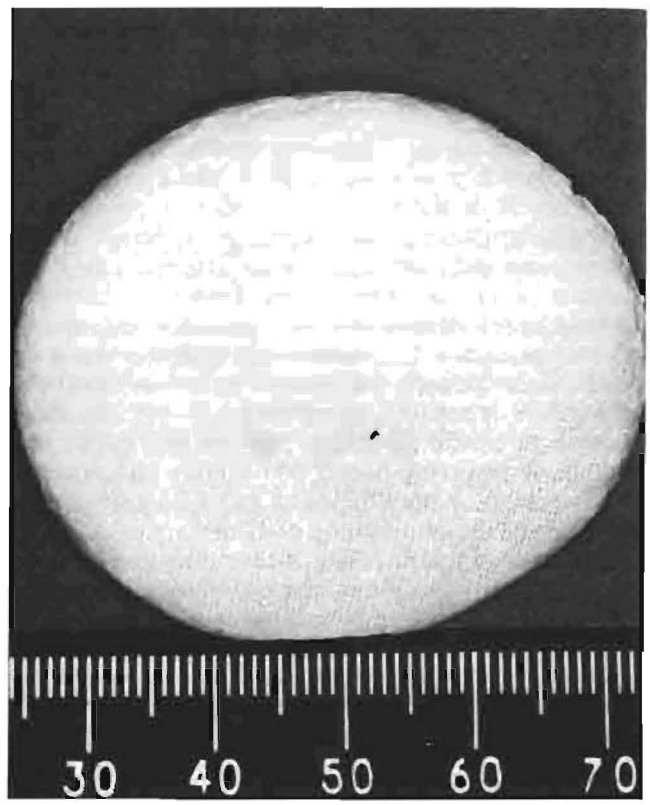

Fig. 1. Bladder calculus removed from an 8-year-old Beagle Bitch.

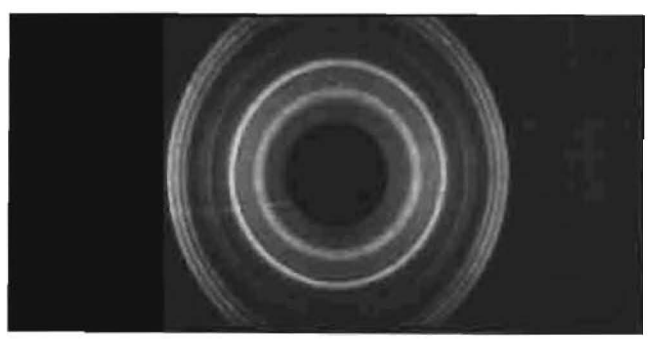

Fig. 2. X-ray diffraction pattern of sample from centre of stone. The spacings and intensities of the lines (reflections) together constitute the "fingerprint" of the crystalline substances present.

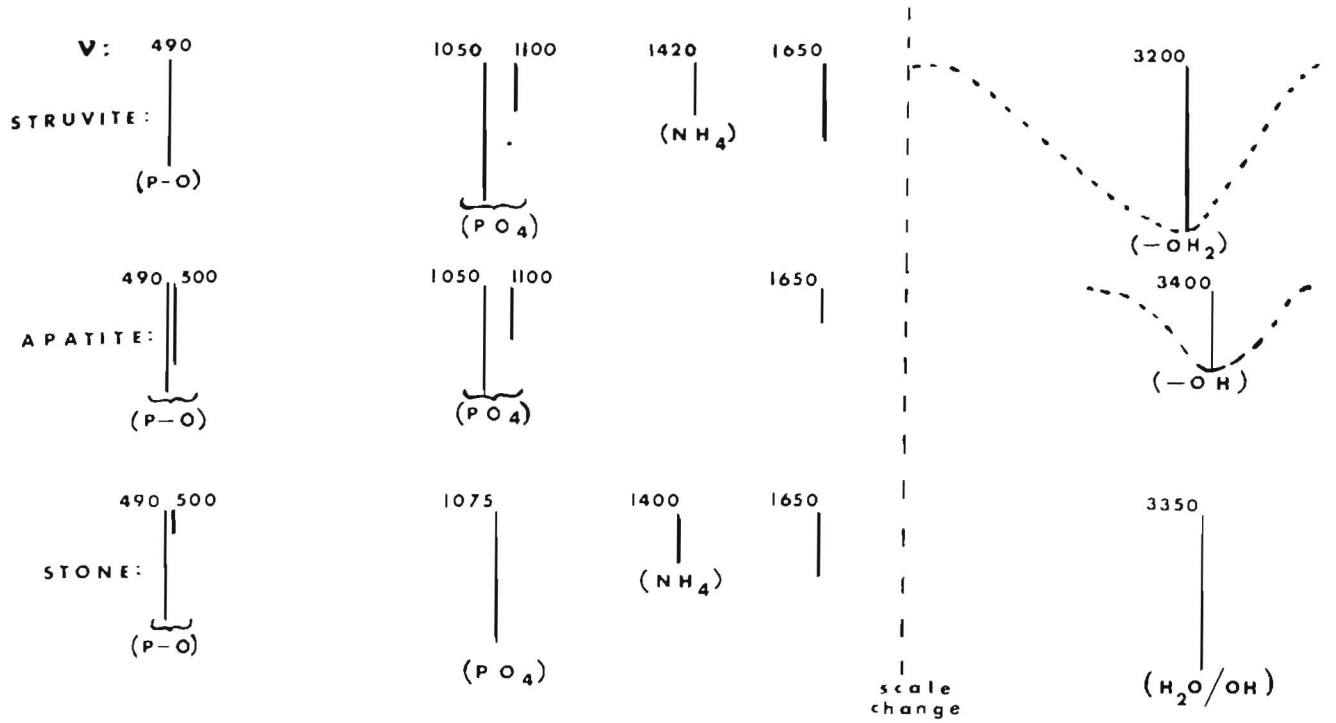

Fig. 3. Schematic representation of the *major* infrared bands of two standards (struvite and hydroxyapatite) and the stone.

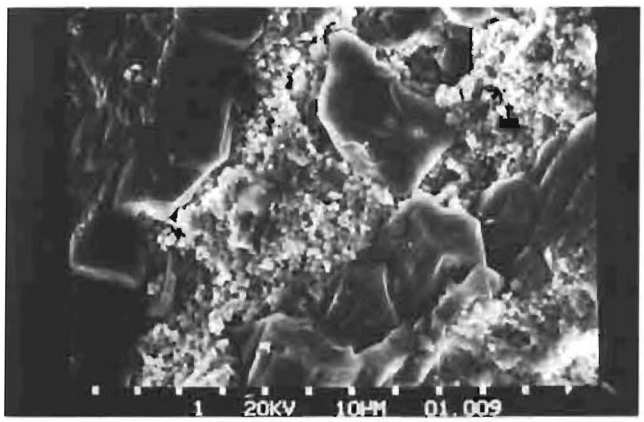

Fig. 4. SEM micrograph showing large struvite crystals interspersed with deposits of hydroxyapatite.

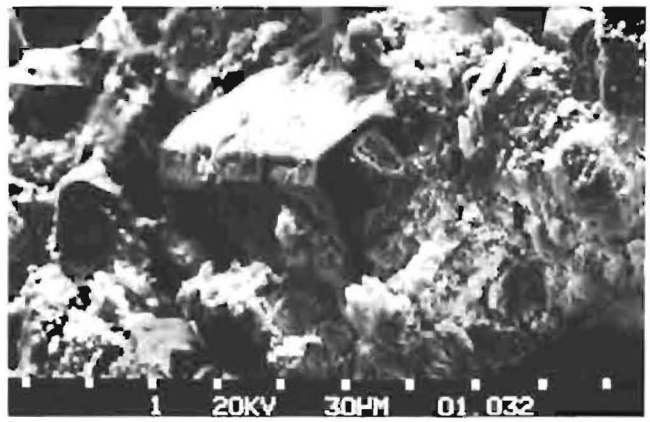

Fig. 5. SEM Micrograph of a single struvite crystal surrounded by hydroxyapatite deposits and other struvite crystals.

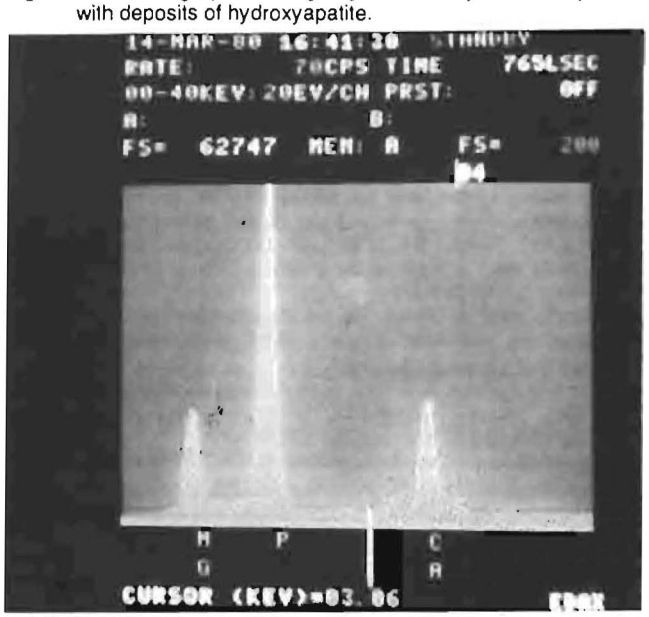

Fig. 6. X-ray energy dispersive analysis display of the entire area imaged in Fig. 5. The P peak is due to the presence of both struvite and hydroxyapatite, while Mg is due to the former only and Ca to the latter only.

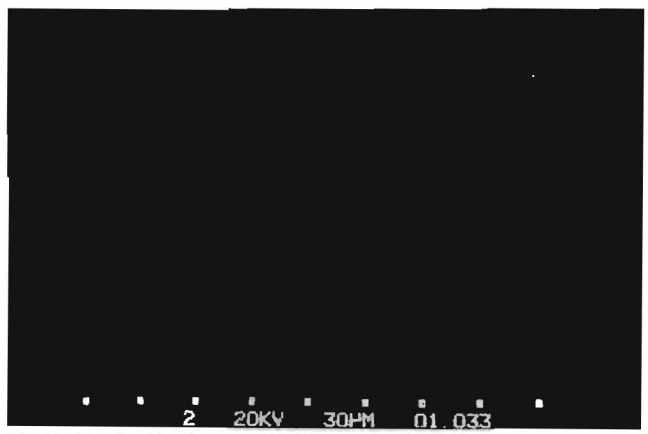

Fig. 7. Mg distribution image of the area shown in Fig. 5. The white dots correspond to the presence of Mg (and hence struvite).

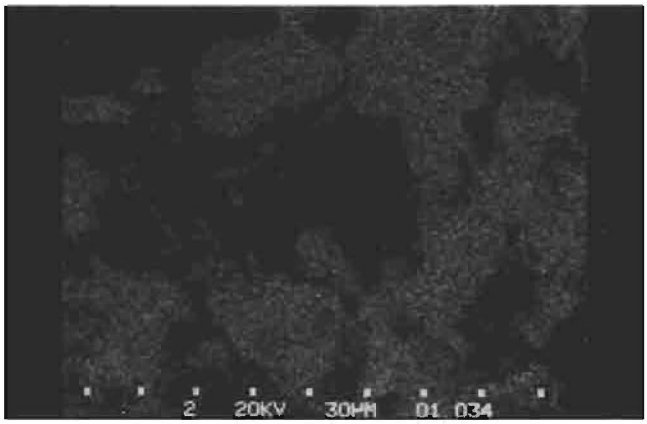

Fig. 8. Ca distribution image of same area showing the location of the hydroxyapatite deposits.

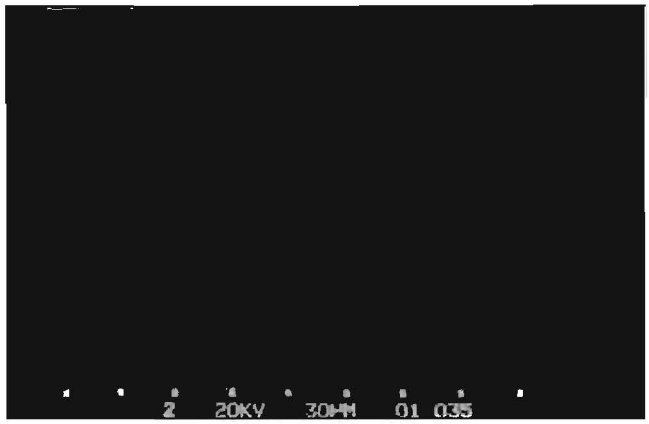

Fig. 9. P distribution image. Those regions in which there is no P, Ca or Mg indicate the presence of holes or organic constituents.

calibration was adopted thereby permitting correction of the Mg absorbence curve[9]. From this and the Ca absorbence curve the concentration of the two metals and hence of struvite and hydroxyapatite were determined.

DISCUSSION

The results show that a complete and thorough analysis of the single calculus has been accomplished. As mentioned earlier, x-ray diffraction studies have the serious limitation that components may go undetected for a variety of reasons. However, in this case, the second "finger print" method, infrared spectroscopy, confirmed the initial assignment and failed to detect the presence of a third constituent. Examination in the scanning electron microscope together with energy dispersive analysis provided the opportunity for the study of morphology and ultrastructural inter-constituent relationships. The quantitative results show remarkable agreement and lend confidence to the techniques both singularly and collectively.

Because separation into 2 discrete layers (struvite and hydroxyapatite) did not occur in the density column, it may be concluded that these 2 components were intimately associated within the stone. This suggests that sequential deposition of pure components from the urine was not the major means of crystal aggregation and is further borne out by the fact that struvite and hydroxyapatite deposits were observed freely interspersed with one another at the ultra-structural level.

Presence of bacteria in the urinary passages is a predisposing factor in the formation of calculi. The occurrence of struvite in animal as well as human urinary stones has been taken as indicative of infection by urea-splitting organisms. Whether it can occur in sterile urine is not known. However, it is the usual constituent of feline calculi and urethral plugs responsible for urinary obstructions in male cats[10]. The character of these plugs is quite different from the calculi normally encountered and can be broken down by simple digital pressure. It is interesting to note that while these struvite deposits can form in bacterially sterile urine, viruses are thought to be associated with their formation[11].

In conclusion, this study has shown that *total* analysis of animal calculi is possible by adoption of a multiple technique approach. It is perhaps unnecessary to employ all the techniques presented here. For example, only one of x-ray diffraction and infrared spectroscopy need be applied to identify the constituents present while again only one of the 3 quantitative procedures should suffice for this aspect of the investigation. These, coupled with scanning electron microscopy studies, should provide the veterinary scientist with more than sufficient data to formulate an accurate diagnosis and mode of treatment on the one hand and to gain some insight into understanding stone formation mechanisms on the other.

ACKNOWLEDGEMENTS

This work was supported by a grant from the University of Cape Town. The SEM and XRF studies were conducted in the Electron Microscope Unit and Department of Geochemistry, respectively. All other work was carried out in the School of Chemistry and Department of Medical Biochemistry. One of us (K.M.) would like to thank the CSIR for the award of a postgraduate research bursary.

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CASE REPORT GEVALVERSLAG

A MYCOBACTERIOSIS IN A SHEEP RESEMBLING PARATUBERCULOSIS (JOHNE'S DISEASE)

S.J. NEWSHOLME* and J.M. PLETCHER†

ABSTRACT: Newsholme S.J.; Pletcher J.M. **A mycobacteriosis in a sheep resembling paratuberculosis (Johne's disease).** *Journal of the South African Veterinary Association* (1981) **52** No. 2 143–145 (En) Veterinary Research Institute, 0110 Onderstepoort, Rep. of South Africa.

In a sheep which was euthanased because of severe emaciation and weakness, slight thickening of the ileum was seen grossly. Microscopically there was a granulomatous ileitis with obliterative lymphangitis and lymphangiectasia. Granulomatous lesions were also present in the liver and some mesenteric lymph nodes. Large numbers of acid-fast bacilli were present within epithelioid macrophages in the lamina propria of the ileum.

Although the identity of the *Mycobacterium* spp. involved was not established, the possibility of paratuberculosis is discussed. The apparent rarity of this disease in sheep in South Africa is considered. Particular attention is drawn to the absence of diarrhoea in this case, to the slightness of the gross changes and to the importance of submitting material for mycobacterial culture.

INTRODUCTION

A sheep born and raised at the Veterinary Research Institute, Onderstepoort, developed severe emaciation and was euthanased. Although a definitive diagnosis was not made, gross and microscopical evidence suggested that the animal may have been suffering from paratuberculosis (Johne's disease). This report is presented in view of the apparent rarity of ovine paratuberculosis in the Republic of South Africa.

HISTORY

A 2-year-old Dorper ram, one in a group of 5 yarded sheep at the Veterinary Research Institute, Onderstepoort, developed severe weight loss over an undetermined period. The ram became extremely thin and so weak that it was unable to stand. The other sheep in the group remained in good condition. The ram was euthanased by intravenous injection of pentobarbitone sodium. Twelve days prior to euthanasia of the ram, all sheep in the group had received a single, subcutaneous injection of Freund's complete adjuvant, prepared according to standard procedures.

All the sheep had been bred in the sheep yards at Onderstepoort where they had remained since birth. None of them had been subjected to any previous experimental studies and the single injection of Freund's complete adjuvant had been the only experimental intervention.

GROSS CHANGES

Severe emaciation was evident at post-mortem examination. Slight, diffuse thickening of the ileal mucosa was noted, involving the segment from approximately mid-ileum to the ileo-caecal valve. No other gross changes were seen in the carcass other than those referable to the method of euthanasia. Digestive tract contents appeared normal in colour and consistency with no evidence of diarrhoea.

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Specimens of ileum, taken from different levels, mesenteric lymph nodes and liver were fixed in 10 % buffered formalin.

MICROSCOPIC CHANGES

Paraffin sections of the fixed tissues were prepared and stained with haematoxylin and eosin (HE) and by the Ziehl-Neelsen method[4].

In the affected portion of the small intestine the villi were blunted (Fig. 1) and villous epithelium was cuboidal rather than columnar. Intestinal crypts were irregular and some were dilated and contained necrotic debris. A diffuse, granulomatous inflammatory process was evident, characterized by profuse accumulations of epithelioid macrophages in the lamina propria (Fig. 2) and an obliterative, granulomatous lymphangitis with concomitant lymphangiectasia (Fig. 3). The lymphatic lesions were most prominent in the submucosa which was oedematous. Occasional giant cells were present in the lamina propria and in the lymphatics (Fig. 4).

Microscopic lesions were also observed in the liver and mesenteric lymph nodes. A multifocal, granulomatous hepatitis was evident with microgranulomata mainly in the portal and periportal areas. Granulomatous lymphadenitis was noted in many of the mesenteric lymph nodes. Large numbers of epithelioid macrophages were present in both cortex and medulla as well as in the subcapsular sinuses of the affected nodes. Ziehl-Neelsen staining revealed numerous acid-fast bacilli situated within the cytoplasm of epithelioid macrophages in the lamina propria. A few acid-fast bacilli were also found within macrophages in the hepatic granulomata and mesenteric lymph nodes.

DISCUSSION

Clinical and pathological features of this case are similar to those reported previously for ovine paratuberculosis. Progressive emaciation, with or without diarrhoea, gross thickening of the wall of the ilem[3 5 9 10 11 13], granulomatous ileitis with atrophy of the villi and crypts[3 5 9 10 13], obliterative lymphangitis[5 9 10 13] as well as granulomatous involvement of the mesenteric lymph

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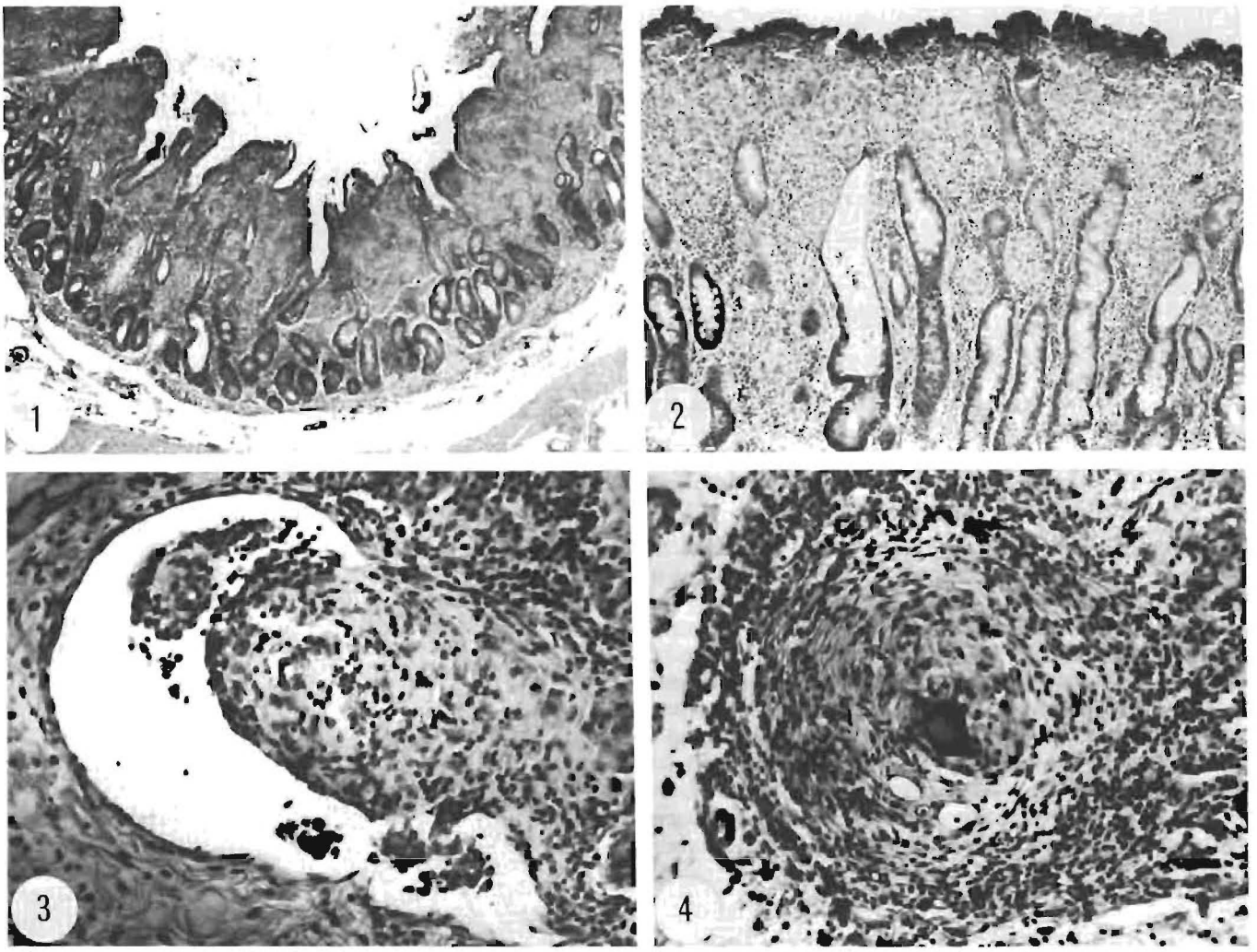

Fig. 1 Ileum, showing mucosal thickening and blunted villi.

Fig. 2 Intestinal crypts are slightly disrupted by a diffuse infiltration of epithelioid macrophages in the lamina propria. Several crypts are dilated.

Fig. 3 A lymphatic vessel of the submucosa showing marked dilatation and partial obliteration of its lumen by infiltrating macrophages.

Fig. 4 Complete obliteration of a submucosal lymphatic vessel by granulomatous inflammatory cells, including a multinucleated giant cell in the centre.

nodes[3 5 9 10 13] are all described as features of this disease in sheep. The granulomatous hepatitis observed in this case is not generally associated with ovine paratuberculosis but is well documented for the disease in cattle[2 6].

The presence of numerous acid-fast bacilli within macrophages in the lamina propria adds further support to a diagnosis of ovine paratuberculosis. However, as no attempt was made to culture the organism from affected tissues, a definitive diagnosis has not been made. A *Mycobacterium* spp. distinct from *M. paratuberculosis* might have produced the lesions observed. The possible influence of the previous injection of Freund's complete adjuvant on the development of the lesions must also be considered. Since Freund's complete adjuvant contains no viable bacteria, it is most unlikely to have constituted a source of infection. A possible potentiating effect of Freund's complete adjuvant upon a pre-existing mycobacterial infection cannot be ignored. However, we consider that the interval of 12 d from administration of adjuvant to euthanasia was too short for the development of the advanced granulomatous lesions seen in this case.

Although it was not confirmed, accumulated evidence suggests to us that ovine paratuberculosis is the most appropriate diagnosis.

Severe emaciation in the absence of diarrhoea was an interesting feature of this case. One explanation for this could be a net loss of protein through the affected segment of the ileum. Increased loss of plasma proteins into the intestine has been demonstrated in bovine paratuberculosis[7 8] and also in experimental ovine paratuberculosis[1]. Protein losing enteropathy in paratuberculosis and in certain other conditions in cattle has been related to intestinal lymphangiectasia[7]. Although serum protein levels were not measured in the present case, intestinal lymphangiectasia was certainly present.

If the aetiological agent in this case was indeed *M. paratuberculosis,* this represents the first record of paratuberculosis in an indigenous sheep in South Africa. The only other record of ovine paratuberculosis in South Africa[12] concerns a case of an imported sheep which may have acquired its infection prior to importation. Although it was described as a case of ovine paratuberculosis, culture of the causative organism was not reported.

This case is reported to direct attention to the possible occurrence of ovine paratuberculosis in South Africa. It conforms to previous observations that intestinal gross changes may be slight[5 10 13] and easily overlooked in this disease. Practitioners should be aware that diarrhoea is not a constant feature of ovine paratuberculosis and should include this disease in their differential diagnosis when confronted with emaciation in adult sheep. Failure to establish a definitive diagnosis in this case illustrates the importance of submitting appropriate material for mycobacterial culture where paratuberculosis is suspected.

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ABSTRACT: Van Wyk, J.A., Gerber, H.M. & Groeneveld, H.T., 1980. A technique for the recovery of nematodes from ruminants by migration from gastro-intestinal ingesta gelled in agar: large-scale application. *Onderstepoort Journal of Veterinary Research,* 47, 147–158 (1980).

A gelled-agar technique for worm recovery was adapted to facilitate the recovery of larval and adult nematodes from the total ingesta of large numbers of sheep. The technique was also used to recover nematodes from 4 calves.

In one trial involving 120 sheep, 100% of 2 013 4th stage larvae (L4) and 92,1% of 134 205 adult *Haemonchus contortus* migrated from the agar preparations. Highly significantly more male than female worms failed to migrate.

Using 1 × 1/10 aliquot to estimate the numbers of worms that failed to migrate from the agar, the mean error in the total worm count (worms that migrated plus those that failed to migrate) per sheep was 2,2%; with an examination of 2 × 1/10 aliquot the error was 1,7%.

We concluded from this that the gelled-agar method may be of value for quantitative worm recovery, for example, in anthelmintic tests.

In a second trial, 98,5% of 17 056 L4 and adult nematodes of 5 genera migrated from the ingesta of 4 calves and 96,4% of 62 597 L4 and adult nematodes of 9 species from the ingesta of 15 sheep.

In general, L4 migrated slightly more efficiently than adult worms. In sheep and, to a lesser extent, in calves, *Haemonchus spp.* did not migrate as efficiently as the other genera such as *Ostertagia, Trichostrongylus, Nematodirus, Oesophagostomum, Marshallagia* and Chaberia.

ABSTRACT: Van Wyk, J.A. & Gerber, H.M., 1980. Survival and development of larvae of the common nematodes of ruminants after long-term cryopreservation and investigation of different routes of infestation. *Onderstepoort Journal of Veterinary Research,* 47, 129–136 (1980).

Exsheathed infective larvae (L3) of 16 species of nematodes were tested for infectivity in either sheep or cattle after they had been frozen and stored in 0,09% NaCl solution in the gas phase of liquid nitrogen for periods of up to 59 months.

A mean of >90% of the L3 of *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis, Nematodirus spathiger, Oesophagostomum columbianum* and *Chabertia ovina* of sheep and *Haemonchus placei, Ostertagia ostertagi, Cooperi spp. (C.pectinata* and *C. punctata), Nematodirus helvetianus* and *Oesophagostomum radiatum* of cattle was alive when they were thawed, after having been frozen for 52–59 months.

These L3 as well as those of *Marshallagia marshalli* and *Trichostrongylus falculatus,* which had been frozen for 30–33 months, were infective to sheep or cattle when dosed *per os* or inoculated into the abomasum or the duodenum. Thawed *Dictyocaulus filaria* L3, frozen for 31 months, developed poorly when injected intravenously into sheep.

This appear to be the first report showing infectivity of L3 of *O. circumcincta, T. colubriformis, N. spathiger* and, possibly, of *O. radiatum* by the oral route after cryopreservation in liquid nitrogen.

CASE REPORT **GEVALVERSLAG**

AMMONIUM ACID URATE CALCULI IN A CROSS-BRED YORKSHIRE TERRIER

R. RUBINSTEIN*, E.H. HARLEY†, and J.W. ROUSSEAU‡

ABSTRACT: Rubinstein R.; Harley E.H.; Rousseau J.W. **Ammonium acid urate calculi in a cross-bred Yorkshire Terrier.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 147–149 (En) Department of Bacteriology, Medical School, 7925 Observatory, Rep. of South Africa.

The history, clinical signs and pathological findings are described in a cross-bred Yorkshire Terrier which developed renal failure subsequent to the development of renal calculi. These calculi were found to consist of ammonium urate, a rare form of calculi in non-Dalmatian dogs.

INTRODUCTION

Urate stones in the urinary tract are relatively rare in dogs accounting for between 6 and 8 % of the total calculi studies[4 7]. Phosphate, cystine and oxalate stones are much more common. The above figures include Dalmatians, and the incidence of urate stones for non-Dalmatians is even lower at 2 %[7]. Most canine stones have been found in the urethra and/or bladder. Only between 2 and 6 % of stones are found in the renal pelvis[4 9]. In contrast to man, where uric acid renal stones consist of the protonated (undissociated) form, in Dalmatians they are typically ammonium urate[7]. In young dogs, urolithiasis is rare, stones usually occurring at 4 to 10 years with a peak at 6 to 7 years[10]. We describe here the occurrence of ammonium urate stones in the renal pelvis of a young cross-bred Yorkshire Terrier. This unusual pathology (both of stone type and site) caused problems in diagnosis resulting in prolonged illness over a 3-year period.

CASE HISTORY

The animal involved was a male cross-bred Yorkshire Terrier. He was vaccinated at 7½ weeks of age for distempter, hardpad and leptospirosis. Five days later he developed sarcoptic mange which was treated with Rotonone suspension and weekly lime sulphur baths, despite which recovery was prolonged. At 9 months of age he developed malaise, showing slowing of movements, apathy and mild pyrexia. The kidneys were tender on palpation. A clinical diagnosis of urinary-tract infection was made and he was treated with antibiotics. A few months later a β haemolytic streptococcal tonsilitis, confirmed bacteriologically, was treated with antibiotics. Thereafter, he had periods of apparent good health alternating with episodes usually typified by vomiting and diarrhoea, occasionally with symptoms of lassitude. These episodes were treated with a variety of antibiotics and changes of diet, prescribed in case he had sensitivity to certain foods. Despite this his health deteriorated, with vomiting on most days, usually in the morning, increasing discomfort, salivation and occasional enuresis with strongly smelling urine. No conclusive diagnosis was reached at this time.

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At 3 years and 2 months of age, a catheter specimen of urine showed several small round smooth grey stones. Coliform organisms resistant to ampicillin were isolated. Attempts to treat the infection with nitrofurantoin (Furadantin, Smith Kline and French (Pty) Ltd) were curtailed by vomiting. Cortisone was prescribed for a presumptive alternative diagnosis of prolapsed intervertebral disc. There was a short-lived improvement.

A month after the catheterization he was treated with N-(2' chloro 4 nitrophenyl)-5-chloro-salicylamide, 76,92 % (Lintex, Bayer) for tapeworm *(Dipilidium caninum)*, but this was followed in a few hours by collapse and extreme weakness. Radiography showed a grossly enlarged left kidney. Laparotomy was performed (September 1977) and a stone was found to be obstructing the left ureter. The kidney was therefore removed and three large stones were found occupying the renal pelvis.

The post-operative course was complicated by temporary paralysis of the hindquarters, thought to be due to an embolic episode, and later by a prolonged period of discomfort with rigors and another episode of weakness. However, this eventually resolved after treatment with cotrimoxazole (Bactrim, Roche) followed by cephalothin (Ceporacin, Glaxo) and metronidazole (Flagyl, May Baker). On the basis of the stone analysis he was treated with a low purine diet and allopurinol (Zyloprim, Wellcome), 50 mg/d. At just over 4 years of age he began to show increasing periods of lethargy and discomfort. He was eventually treated for persistent tapeworm infection with arecoline acetarsol (Cestarsol, May Baker) followed by an enema. However, lethargy and discomfort increased in the course of the next 3 days, and despite injection of chloramphenicol, vitamin B_{12} and methionine, collapse and convulsions followed. The blood urea nitrogen was found to be 11,62 mmol/ℓ and euthanasia was performed.

PATHOLOGICAL FINDINGS

Gross morphology

1st kidney (removed at laparotomy) showed mottled appearance with focal abscesses. Three large stones were found in the renal pelvis.

2nd kidney (removed post-mortem) was of normal size and appearance except for slight evidence of scarring. Cortex was of normal thickness. Several large stones were found in the renal pelvis.

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Histology

1st kidney – Examination of sections (Fig. 1) revealed acute pyelonephritis. The interstitial tissues were infiltrated with large numbers of polymorphonuclear leukocytes. Although there was focal destruction of nephrons by the inflammatory process, there was no evidence of widespread tubular necrosis. An occasional glomerulus showed focal mesangial scarring, but there was no evidence of glomerulonephritis. Many of the glomeruli showed dilatation of Bowman's space in keeping with hydronephrosis. Staining with periodic acid-Schiff reagent, showed that there was no thickening of the basal membrane of the glomeruli; an immunological disorder was therefore considered to be unlikely.

2nd kidney – There was evidence of chronic pyelonephritis, with chronic inflammatory cells present in areas of interstitial fibrosis.

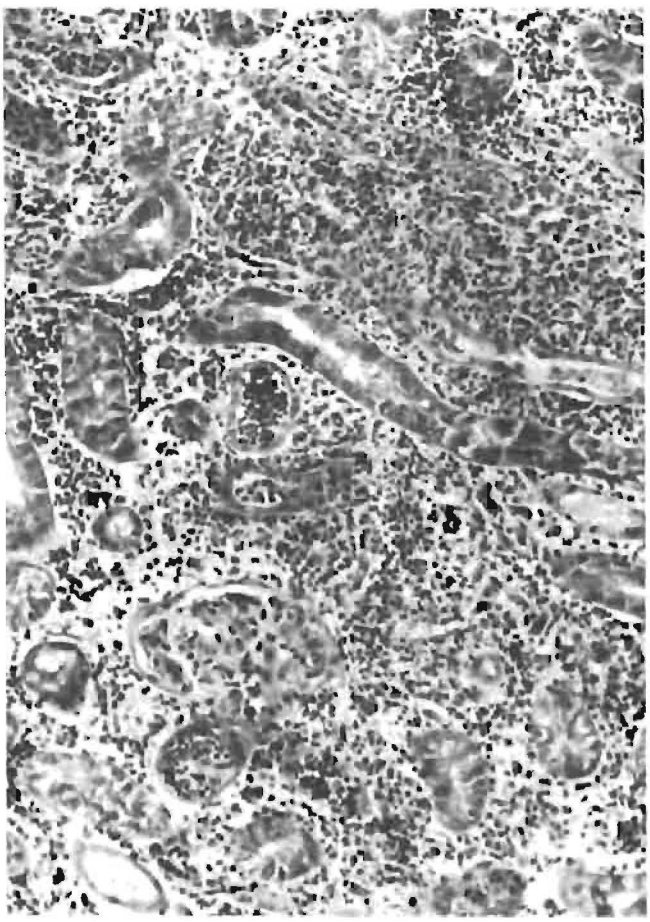

Fig. 1. Haematoxylin and eosin section of first kidney showing acute pyelonephritis.

CHEMICAL INVESTIGATIONS

(i) Blood urea nitrogen was generally normal or only slightly elevated, until the terminal episode.

(ii) Uric acid in serum or urine was determined spectrophotometrically by measurement of the decrease in absorbance at 293 nm after addition of uricase. Serum uric acid post-nephrectomy was only 0,030 mmol/ℓ. This compared with a mean of 0,074 ± 0,066 (2 × S.D.) for 99 control mongrel dogs (Briggs and Harley, Dept. Chem. Path. U.C.T., 1980 unpublished observations). Urinary urate at this time was 2,7 mmol/ℓ.

STONE ANALYSIS

The stones were irregular in shape and bright blue in colour. Three methods were used to analyze the composition of the stone:

(a) Murexide test[11]. This gave the characteristic blue colour showing the presence of urate.

Fig. 2. X-ray diffraction powder photograph of ammonium acid urate found in urinary calculi. In this method, a powdered specimen of the stone is exposed to a beam of monochromatic X-rays. These are diffracted by the various planes of atoms and are recorded on film as a series of arcs of varying intensity. The pattern in characteristic of any given organic compound and therefore provides a fingerprint for its identification[12].

(b) X-ray diffraction[12]. The spacings and relative intensities of the lines obtained (Fig. 2) correspond almost exactly to the standard powder pattern for ammonium acid urate[8].

(c) Infra-red Spectroscopy[13]. Minor constituents may be missed using X-ray diffraction, and therefore infrared studies were performed. This provides a record of the wavelengths at which a substance absorbs electromagnetic radiation in the infrared. It is characteristic of many organic or inorganic compounds and can provide a fingerprint for its identification. This method has been used to identify minor constituents present in amounts as low as 1 %. All the peaks in the spectrum obtained corresponded to the standard ammonium acid urate spectrum. No minor constituents were detectable.

BACTERIOLOGY

August 1977 (pre-nephrectomy): *Escherichia coli,* sensitive to nitrofurantoin, was isolated from a catherer specimen of urine (pH 5). October 1977 (1 month post-nephrectomy): *E. coli* was again isolated from a mid-stream urine specimen (pH 6).

November 1977: The urine was sterile and no significant bacteriuria was found thereafter at regular monthly examinations, the pH remaining at approximately 6.

DISCUSSION

In 1916, Benedict[2] discovered that the Dalmatian was unique in its ability to excrete proportionally as much uric acid per day as man. Dalmatians therefore develop more urate stones than other breeds. A few cases in other breeds have been reported[9]. Friedman and Byers[5] suggested that the cause of the high urinary excretion of uric acid in Dalmatians is a renal tubular reabsorptive defect, but more recent evidence suggests that the predominant defect is a lowered rate of hepatic conversion of urate to allantoin, despite apparently normal uricase levels. Porter[7] showed that other breeds can excrete urates with urinary concentrations within the lower range observed for the Dalmatian but only when simultaneously excreting high concentrations of allantoin. The range of uric acid concentrations they found ranged from 3 to 13 mmol/ℓ. Urate calculi are described as being small and spherical. Stones with this appear-

ance were seen in a catheter specimen of urine pre-nephrectomy. However, the stones found in the renal pelvices in the present case were large, irregular in shape and bright blue in colour. Stones of this size, shape and colour have not been described before. No methylene blue was used as a urinary antiseptic, and the unusual colour is at present unexplained. There are two possibilities of the cause of the stone formation in this animal. Either he had a primary genetic defect in purine metabolism or transport, or the stone formation was secondary to urinary tract infection. Blood urate levels were low normal, but urinary urate concentration was similar to that reported for Dalmatians, levels in mongrel dogs usually being about 5-fold lower[6]. However, in the absence of full daily urine collections for balance studies, too much emphasis cannot be placed on this urinary urate figure. Urinary tract infection may be instrumental in causing ammonium urate precipitation, either by alkalinizing the urine or by disturbing the colloidal properties of urine which normally delay crystal formation in supersaturated urate solutions[9]. In this context it is perhaps of relevance that herpes virus infection can induce urolithiasis in the cat[3].

The main point of interest in the present case is the demonstrations of ammonium urate stones of an unusual type and site in a non-Dalmatian dog, and to emphasize the mode of presentation and symptomatology. This may assist future diagnosis and show whether urate stones are, in fact, as uncommon as previously considered.

ACKNOWLEDGEMENTS

We are most grateful to D. Blake for technical assistance and to Prof. E.G. White and Dr. A. Rose for helpful discussions. Dr. A.L. Rodgers kindly performed the X-ray diffraction studies.

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ABSTRACT: Littlejohn, A. 1980 Studies on the physiopathology of chronic obstructive pulmonary disease in horses. I. Clinical signs. *Onderstepoort Journal of Veterinary Research,* 47 159–162 (1980).

Twenty cases of chronic cough originating in the lung and associated with loss of performance were clinically examined. The physical signs observed were compared with those observed in a control series of 38 clinically normal horses.

Reduced work tolerance, coughing for more than 3 months and abnormal pulmonary sounds (râles) were primary signs of chronic obstructive pulmonary disease (COPD). Forced abdominal expiratory efforts and pumping of the anus were regarded as confirmatory signs.

Neither nasal discharge nor increased marginal distance was found to be a reliable sign of COPD.

The mean respiratory frequency of the COPD subjects, namely 25,4 per minute, was significantly higher than the 16,7 per minute ($P<0,001$) of the 38 normal subjects.

ABSTRACT: Littlejohn, A. & Bowles, Felicity 1980 Studies on the physiopathology of chronic obstructive pulmonary disease in the horse. II. Right heart haemodynamics. *Onderstepoort Journal of Veterinary Research,* 47 187–192 (1980).

Pressure curves obtained by cardiac catheterization of the pulmonary artery, right ventricle and right atrium of 9 horses and ponies with chronic obstructive pulmonary disease (COPD) were compared with those similarly recorded from 6 clinically normal control subjects.

The mean pulmonary peak systolic, pulmonary minimum diastolic and ventricular peak systolic pressures of the COPD subjects were significantly higher ($P<0,01$) than the corresponding mean pressures of the clinically normal control subjects.

The mean pressures calculated from pressure curves obtained from 8 Thoroughbreds in training did not differ significantly from those of the clinically normal subjects not in training.

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CASE REPORT **GEVALVERSLAG**

FATAL HEART FAILURE IN A POODLE DUE TO WIDESPREAD METASTATIC MALIGNANT HAEMANGIOENDOTHELIOMA

G.J. LOUW* and S.J.E.M. VAN SCHOUWENBURG†

ABSTRACT: Louw, G.J.; Van Schouwenburg, S.J.E.M. **Fatal heart failure in a poodle due to widespread metastatic malignant haemangioendothelioma.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 151–153 (En) Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

An elderly male Poodle was presented with haematuria, syncopy, a heart murmur and varying other symptoms which were all treated unsuccessfully. They were linked on post-mortem examination to a widely disseminated metastatic malignant haemangioendothelioma.

CASE HISTORY

An 11-year-old male Poodle was presented in December 1979 for examination because of the observations by the owner of blood-stained urine. This dog had been perfectly healthy up to this point, and had been attended by the veterinarians only for routine examinations and required inoculations. The owner reported that the dog was quite healthy in spite of the haematuria.

CLINICAL SIGNS

The dog had a temperature within the normal range, appeared healthy and lively, and no abnormality could be ascertained on palpation of the urinary bladder. It was decided to leave the dog untreated until something more specific arose.

In April 1980, the dog was again presented for examination because far more blood was then seen in the urine by the owner, occasionally even fresh clots of blood, expecially after excitement or exercise. A diagnosis of cystitis with ulceration of the wall of the bladder was hazarded at, and the animal was treated with Sulpha-antibiotic combinations (Sulphadimethoxine 500 mg tablets, Goldfields). This appeared to afford temporary relief, since no further fresh blood was observed in the urine for a while.

In June 1980 the dog was still very lively and healthy, but the haematuria was considerable. A urine sample demonstrated blood (+) and protein (++) on a Labstix (Ames) strip. It became obvious that there was a haemorrhaging ulcer or malignancy in the bladder. A course of Furadantin (Nitrofurantoin, Smith, Kline & French) was followed for 3 weeks, since there was client resistance to laparotomy and cystotomy. During this time, fresh blood clots were observed in the urine, mainly after hard exercise and barking. Upon clinical examination, the caudal abdominal contents were painful on palpation. A common analgesic was suggested (e.g. acetylsalysilic acid) which was to be administered when the dog seemed uncomfortable.

Two weeks later the dog suffered a "fainting spell" (syncopy) but recovered after a few seconds. When presented at the clinic, a faint heart murmur was audible. The dog still had haematuria. The treatment then involved Lanoxin tablets (Digoxin 0,25 mg, Wellcome), Furadantin suspension and analgesics.

The following days showed a progressive deterioration, involving pyrexia, frequent syncopy, very pale mucous membranes, anorexia, an increasingly loud heart murmur, dyspnoea and bilateral râles in the lungs. Only temporary improvement could be found with Temaril-P tablets (Trimeprazine tartrate 5 mg, Prednisolone 2 mg, Smith, Kline & French) and injections of Betsolan-soluble injectable (Betamethazone 2 mg, Glaxo) and Deltacortril V (Prednisolone 10 mg/mℓ, Pfizer).

At the end of July, the dog exhibited severe respiratory embarrassment and circulatory difficulty, neither of which responded to treatment, so he was referred to the Department of Medicine, Faculty of Veterinary Science, University of Pretoria, where he was briefly examined prior to his sudden collapse. The dog was treated with aminophyllin and Lasix V (Hoechst) but died. An electrocardiogram was performed before the dog died, a section of which is shown in Fig. 1.

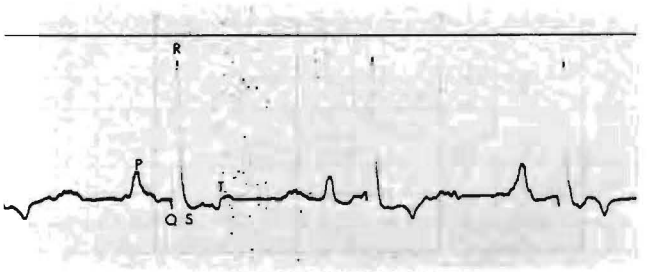

Fig. 1. A section of the electrocardiogram tracing.

The heart rate was 84/min (within the normal range of 70 to 160). Sinus arrhythmia is evident but is considered to be normal. The P-wave has an average duration of 0,04 sec (considered to be normal) and an average amplitude of 0,45 mv (in excess of the normal maximum of 0,40 mv). The latter could indicate right atrial dilation, but in view of the fact that this P-wave sometimes exceeds a duration of 0,04 sec, the dog probably suffered from biatrial dilatation. The PR-interval is 0,16 sec (which exceeds the normal range of 0,06 to

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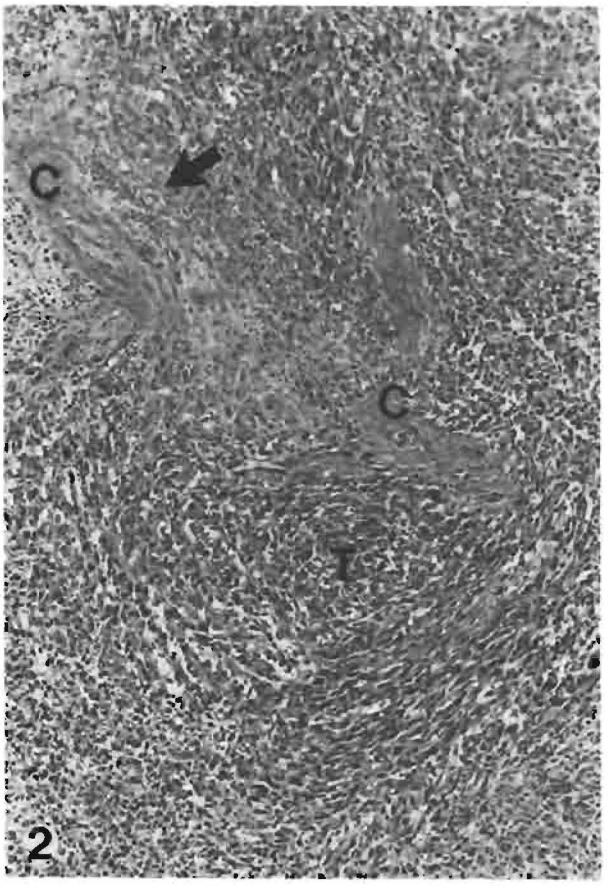

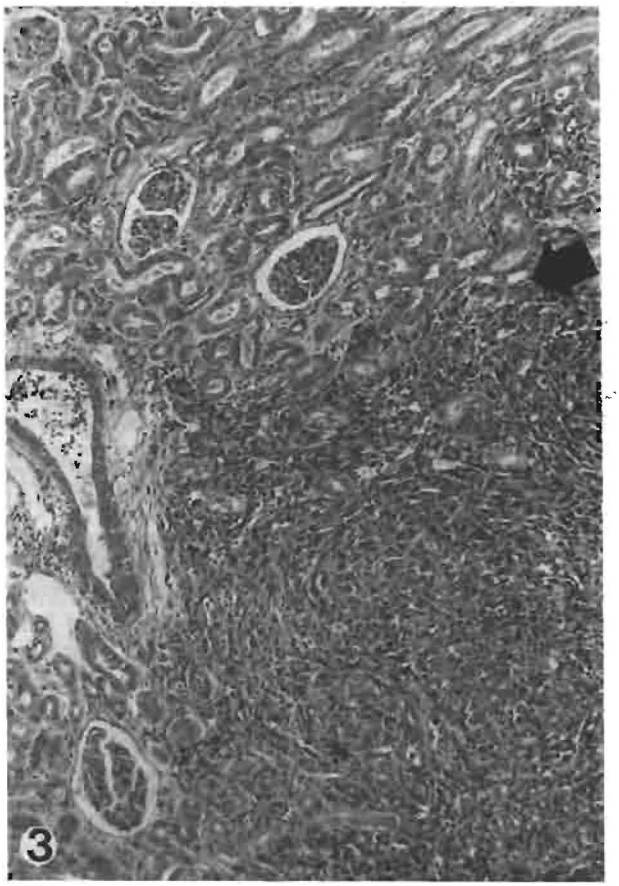

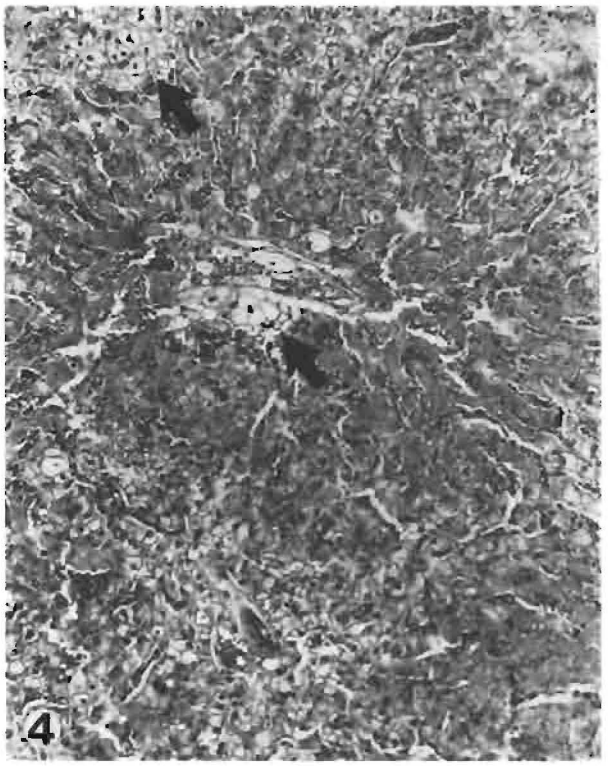

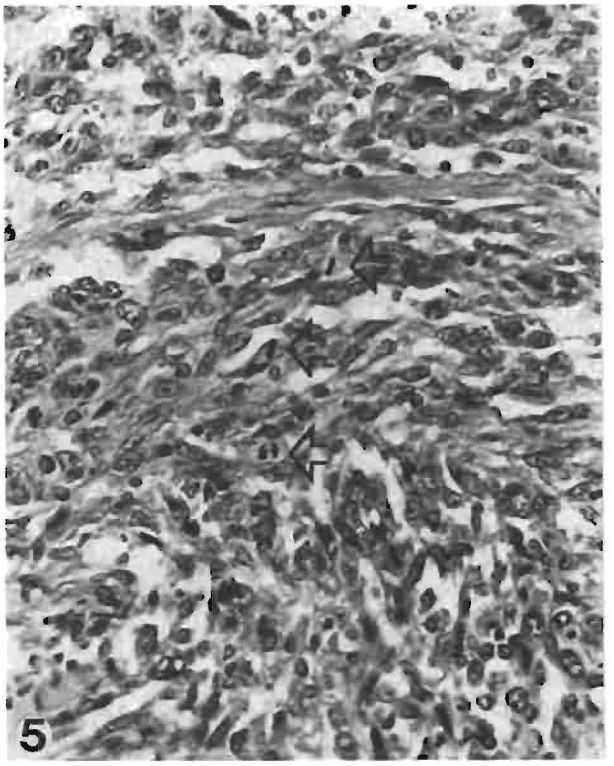

Fig. 2. Tumourous tissue (T) invading the myocardium (C) in an histological section of the right atrial wall. The arrow indicates an area of haemorrhage in the tissue of the haemangioendothelioma. H.E. × 175.

Fig. 3. Neoplastic tissue invading the tissues of the kidney (arrow). H.E. × 175.

Fig. 4. A lobule of the liver, showing degeneration of hepatocytes (arrows) as the tumour metastasises into the liver. H.E. × 250.

Fig. 5. A section of the abdominal wall, showing numerous mitotic figures (arrows) H.E. × 300.

0,13 sec) and is probably a digitalis effect. The R-wave has an average amplitude of 2,4 mv (the normal maximum is 2,5 to 3,0 mv) and an average duration of 0,06 sec (which is the normal maximum)[1]. Although these 2 values are within the normal range for large breeds of dogs, the values are "borderline", and would be suspicious for left ventricular enlargement.

PATHOLOGY

The lungs showed diffuse neoplastic nodules in the parenchyme of all lobes. These nodules were 5–20 mm in diameter, and had blood-filled lumina. The heart had a 20 mm diameter neoplastic nodule in the right atrial wall, and the atrioventricular valves were thickened and misformed by endocardiosis. The liver was enlarged, with focal disseminated fibrous nodules. The kidneys showed white foci 10–30 mm in diameter which were disseminated in the cortex. The mesenterium and mesenterial lymph nodes were affected by these neoplastic nodules. The urinary bladder had 20 mm diameter nodules in it.

Microscopic sections were made of the right atrial wall, lungs, liver, bladder, kidneys, regional lymph nodes and wall of the abdominal cavity, which all showed the presence of malignant haemangioendotheliomas.

Histological sections show immature endothelial cells forming vascular spaces in the tumourous tissues, which contain blood and thrombi. The very cellular areas are difficult to distinguish from fibrosarcomas. In many areas the vascular spaces have ruptured with resultant haemorrhage (Fig. 2). Haemorrhage and necrosis are constant features. The cells vary in size and shape, but are usually elongated, with round or oval nuclei that are hyperchromatic. Mitotic figures are common (Fig. 5). Within the tumourous tissue, connective tissue appears in variable amounts and is difficult to distinguish from neoplastic tissue. There are numerous macrophages laden with haemosiderin, and also considerable infiltration of polymorphonuclear leukocytes[2].

The aetiological diagnosis was metastatic haemangioendothelioma, causing heart failure and death.

DISCUSSION

The original presenting clinical sign was fresh blood in the urine, which must have been considerable, otherwise the owner would not have observed it.
Some of the common causes of haematuria are[3]:

1. Violent exercise
2. Trauma to the urogenital system
3. Iatrogenic trauma (palpation of the bladder or kidneys, catheterization)
4. Urinary calculi
5. Infections of the urogenital system
6. Neoplasia of the urogenital system
7. Systemic disease with haemorrhagic tendencies (thrombocytopaenia, Warfarin poisoning, leptospirosis, systemic lupus erythematosis)
8. Drug-induced (sulphonamides)
9. Parasites of the urinary system
10. Oestrum
11. Renal infarcts

Due to the client's resistance to surgical intervention, an early diagnosis could not be made.

According to Moulton[2], the malignant haemangioendothelioma is a malignant neoplasm of endothelial cells, occurring in dogs mainly, of 9 to 10 years of age, often German Shepherd dogs, and more commonly in males. The animal is presented with a variety of clinical symptoms, depending on the location of the primary and metastatic lesions, as well as the non-specific signs of neoplastic disease. The dog will often show a rapidly developing heart failure, due to extensive neoplastic involvement of the heart and/or haemorrhage into the pericardial sac. Respiratory difficulty is caused by neoplasia and/or haemorrhage into the lung tissues. Acute vascular collapse is caused by rupture of the spleen with severe haemorrhage. The dog is anaemic, showing circulating nucleated erythrocytes and leucocytosis.

Common primary locations are the spleen, right atrium and auricle of the heart, and liver, but since it is a neoplasm of vascular endothelium, it may originate from any site.

The tumour is highly malignant, readily metastasizing and often recurs after surgery. Metastasis occurs early due to its easy access to vascular canals. The lungs are often affected, which will show up as nodules scattered throughout the radiographic field.

Due to the multiple locations involved, it is often difficult to establish the primary site of origin of the metastasizing tumour, and the possibility of a multicentric origin should be considered.

ACKNOWLEDGEMENTS

We wish to express thanks to the following people for their valuable assistance: Dr J. van Heerden, Prof. W.H. Gerneke, Mr J.T. Soley and Dr J.W. Nesbit, of the Faculty of Veterinary Science, University of Pretoria, and Mrs S. van den Hoven for the photography.

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TO THE EDITOR **AAN DIE REDAKSIE**

RESISTANCE TO ORGANOPHOSPHORUS IXODICIDES*

Sir*,

With reference to the two articles by J.A.F. Baker *et al.* in the 49(4) 1978 issue of your journal, the following comments:

1. In the article on "The current status of resistance to organophosphorus ixodicides by the blue tick *Boophilus decoloratus* (Koch) in the Republic of South Africa and Transkei" the criterion used for resistance to dioxathion is that the isolates have a lethal concentration (LC) 99(%) value equal to or greater than that of the reference Holmdene-Berlin strain (p 328). It needs to be stressed, however, that the LC 99 % for the Holmdene strain is 0,006 (p 330, Table 4), which value is only 12 % of, and thus far below, the recommended field concentration of dioxathion.

   In practice, therefore, all the strains illustrated in Fig. 1, (p 328) with a range of susceptibility between that of a susceptible strain and the Holmdene strain, and many of the strains listed with degrees of resistance greater than that of the Holmdene strain, would be controlled by correct dipping, i.e. would not be resistant in the field.
2. In the article on "Resistance to certain organophosphorus ixodicides in the Bont tick *Amblyomma hebraeum* Koch, in the Republic of South Africa and Swaziland", the authors consider as resistant those isolates "for which a history of resistance to dioxathion and chlorfenvinphos in the field is available, and for which LC 99(%) values of 0,03 were obtained". In both cases the recommended field strength of these ixodicides is 0,05 % and isolates showing LC 99(%) values of 0,03 would thus be susceptible to field strengths of the dips concerned.
3. The statement concerning amitraz at the end (p 340) of the latter article is not supported by any factual evidence in the article or reference to the literature, and therefore seems out of place.
4. Care should be exercised when making use of the concept "Factor of Resistance (FOR)" in which all isolates are compared with a particular highly susceptible reference strain. There is a distinct possibility that all isolates showing a FOR greater than the reference strain may be incorrectly interpretated as presenting a practical problem of field resistance to the ixodicide in question.
5. The recommended field strength of dioxathion (0,05 %), for example, will give a value of 50 on the FOR scale when related to the LC 99(%) of the susceptible reference *Amblyomma hebraeum* strain used in the article on resistance in this species of tick. The FOR value of 5,1 for the Ubombo isolate (p 340) would therefore be insignificant and would represent, if anything, a low level of developing resistance.

A.M. Spickett
for Director: Veterinary Research Institute
0110 Onderstepoort.

Sir,

I comment as follows on Mr A. Spickett's letter regarding the interpretation of the results presented in the articles, "The current status of resistance to organophosphorus ixodicides by the Blue tick, *Boophilus decoloratus* (Koch) in the Republic of South Africa and Transkei" and "Resistance to certain organophosphorus ixodicides in the Bont tick, *Amblyomma hebraeum* (Koch) in the Republic of South Africa and Swaziland" published in Volume 49 1978 issue of your journal.

1. The history of the Holmdene strain of *Boophilus decoloratus* having an LC 99(%) value *in vitro* of 0,006, showed that treatment with the recommended field concentration of dioxathion failed to control these ticks. It follows that other collections of *B. decoloratus* having a response *in vitro* to this ixodicide similar to that of the Holmdene strain could be expected to behave in a like manner in the field. Thus, quoting from the relevant article, "Isolates considered as dioxathion resistant are those having LC 99(%) values equal to, or greater than, that for the Holmdene (=Berlin) strain".

   In the absence of data to the contrary, the degree of control obtained in the field by dioxathion against those strains of *B. decoloratus* showing a range of suseeptibility between that of the susceptible reference strain and the Holmdene (=Berlin) strain, remains open to conjecture. The authors did not imply that such strains would show a practical problem of field resistance to ixodicides.
2. Both these above factors are also pertinent to the authors' determination of field resistance to dioxathion and chlorfenvinphos in *Amblyomma hebraeum.*
3. Comparisons of field isolates were made with reference strains maintained at this laboratory for five years. The *B. decoloratus* strain, from the Pietersburg district, Transvaal, is resistant to arsenic, lindane, toxaphene and DDT. The strain of *A. hebraeum,* from the Mapumalanga district, KwaZulu, is resistant to arsenic. Neither strain can be considered highly susceptible.
4. The success of amitraz in controlling field strains of organophosphorus-resistant *A. hebraeum* is supported by detailed case histories (D.A. Davis – Personal communication – 1980) and by general user acceptance of this ixodicide in problem areas.

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*Editor's note: This letter originally appeared in volume 51, page 67, of this Journal and because of the long delay between its publication and the publication of the reply of Mr J.A.F. Baker which appears below, it has been reprinted.

0038-2809/81/02/0154

CORRELATION OF CHANGES IN BLOOD CHEMISTRY WITH PATHOLOGICAL CHANGES IN THE ANIMAL'S BODY: II ELECTROLYTES, KIDNEY FUNCTION TESTS, SERUM ENZYMES, AND LIVER FUNCTION TESTS

LEA STOGDALE*

ABSTRACT: Stogdale L. **Correlation of changes in blood chemistry with pathological changes in the animal's body: II Electrolytes, kidney function tests, serum enzymes, and liver function tests.** *Journal of the South African Veterinary Association* (1981) **52** No. 2 155–164 (En) Department Medicine, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Rep. of South Africa.

The numerous physiological and nutritional factors which influence the concentration of serum calcium are considered. The causes of hypercalcaemia and hypocalcaemia are briefly discussed, with particular reference to the clinical symptoms and pathology. The effect of the acid-base status on the serum-ionized calcium level is stressed. The causes of changes in the serum concentrations of phosphorus and magnesium are briefly reviewed, along with the abnormalities of lactate, pyruvate, and hydrogen ion concentrations.

The kidney function tests, blood urea nitrogen, serum creatinine, and the renal clearance tests are discussed, with emphasis placed on correlating their results with the findings from repeated urinalyses. The important physiologic influences and pathological processes which result in changes in the concentrations of these parameters are delineated.

The causes of increases in the serum enzymes, alkaline phosphatase, alanine transaminase, asparate transaminase, lactic dehydrogenase, sorbitol dehydrogenase, glutamic dehydrogenase, gamma glutamyl transpeptidase, creatinine phosphokinase, amylase and lipase are discussed.

The changes in serum bilirubin concentration and its components are fully described, with emphasis placed on the correlation of the findings with urinalysis data and the complexities resulting from the numerous pathologic conditions causing jaundice. These conditions are listed for each of the domestic animals. The other liver function tests, bromosulphthalein dye retention or excretion, serum uric acid and blood ammonia concentration are briefly considered. All the tests described are very useful, and frequently essential, in aiding the veterinary practitioner to arrive at a diagnosis and prognosis, but they never replace clinical acumen.

SERUM ELECTROLYTES

Serum calcium exists in 2 major forms; ionized and non-ionized. The ionized portion, normally about 50 % of the total, is readily diffusable across capillary membranes and is physiologically active. The non-ionized portion is bound to albumin, and so is non-diffusable and inactive. The calcium content of erythrocytes is very low. Calcium has many functions in the body, including skeletal structure; muscle contraction; conduction and transmission of nerve impulses; neuromuscular excitability; blood coagulation; capillary and cell membrane permeability; and activation of certain enzymes[1].

The concentration of serum calcium is very precisely controlled by an interaction of the intake and storage and by the controlling influence of various hormones. The important factors influencing the homeostatic balance of calcium are[1,12]:

(a) The level of calcium intake. Most foods, apart from milk and dairy products, and lucerne, are very low in calcium.

(b) The Ca: PO_4 ratio. $Ca_3(PO_4)_2$ precipitates as an insoluble salt within the intestinal tract when the concentration of Ca^{++} and PO_4^{3-} exceeds the solubility product. This is important in carnivores when ingested amounts of phosphate are high, such as in meat and liver. In the intestinal tract of herbivores, formation of $Ca_3(PO_4)_2$ salts is increased by alkaline food (most herbage), which increases the pH within the intestines. $Ca_3(PO_4)_2$ salts are not available for absorption through the intestinal mucosa.

(c) The amount of fat in the small intestines. Fat forms insoluble soaps with calcium. High small-intestinal content of fat occurs with fatty diets; pancreatic exocrine enzyme deficiency due to pancreatic atrophy in dogs, or following recurrent pancreatitis; and with bile duct obstruction.

(d) The amount of oxalate or phytate in the diet of non-ruminants, most commonly in horses. These compounds bind calcium, so making it insoluble.

(e) The efficiency of intestinal absorption of calcium is decreased with diarrhoea; cellular infiltration into the small intestinal wall, for example, lymphocytic-plasmacytic enteritis, eosinophilic enteritis, neutrophilic enteritis and abdominal lymphosarcoma; and by glucocorticoid excess.

(f) Activated vitamin D_3 (1,25-dihydroxycholecalciferol), acting as a steroid hormone, induces the synthesis of a calcium-binding protein, and other proteins, in the intestinal mucosal cells. These proteins are involved in the absorption of intestinal calcium.

(g) Parathyroid hormone in the presence of vitamin D_3 causes resorption of mineral $Ca_3(PO_4)_2$ from the skeletal matrix. This action is slow in onset and is a long-term regulator of serum calcium concentrations. Parathyroid hormone also stimulates the activation of vitamin D in the kidneys. The rate of

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parathyroid hormone release is mainly controlled by the concentration of serum-ionized calcium.

(h) Calcitonin inhibits parathyroid hormone-induced skeletal resorption. Calcitonin release is stimulated by increased concentrations of serum-ionized calcium. It is a short-term regulator of serum calcium concentration and principally serves to prevent post-prandial hypercalcaemia.

(i) Ionized calcium in the serum is the active portion of the total serum calcium. Both the proportion and the absolute concentration of serumionized calcium depend on the amount of serum albumin and the acid-base balance of the animal. When the serum albumin concentration is low, the proportion of ionized calcium will be higher than normal. The amount of calcium which is protein-bound depends on the degree of dissociation of the carboxyl binding sites on the albumin. When the pH of the blood rises (alkalosis), more H^+ will dissociate from these groups, making more negative binding sites available for Ca^{++}. Ionized calcium will therefore be reduced. Alkalosis also decreases intestinal absorption and bone resorption of calcium. In the presence of an acidosis less carboxyl groups will dissociate, leading to an increase in the amount of ionized calcium.

(j) Serum phosphate concentration influences the amount of calcium in the serum directly, by causing precipitation of $Ca_3(PO_4)_2$ in tissues when the Ca: PO_4 solubility product is exceded. Indirectly, the serum calcium concentration is altered by the amount of phosphate in the serum calcium concentration is altered by the amount of phosphate in the serum, through influence on the release of parathyroid hormone and calcitonin, and activation of vitamin D_3.

(k) Calcium is excreted mainly through the kidneys. The rate of excretion is decreased by parathyroid hormone.

There are 4 principal indications for evaluating the serum calcium concentration, namely suspicion of bone disease, paralytic syndromes, convulsions and when a parathyroid gland abnormality is suspected[1]. In practice, clinical laboratories only measure total serum calcium and do not differentiate between ionized and protein-bound calcium.

Hypercalcemia occurs rarely and is usually associated with primary hyper-parathyroidism, excessive administration of activated vitamin D or certain neoplastic conditions. Prolonged hypercalcaemia *per se* causes renal tubular nephrosis with resultant kidney failure[9]. Primary hyperparathyroidism is very rare. It is due to a functional adenoma of mixed epithelial cell types or an adenocarcinoma of the chief cells. This results in bone demineralization and fibrous osteodystrophyl even to the extent of osteitis fibrosa cystica; metastatic calcification, expecially nephrocalcinosis; and frequently urinary calculi composed of calcium and phosphate. Secondary hyperparathyroidism, either due to nutritional imbalance or renal dysfunction, results in serum calcium concentrations within the normal physiologic range[11 12].

When massive amounts of activated vitamin D are administered or ingested, hypercalcaemia results. This causes metastatic calcification, particularly affecting the intima and media of the large arteries (arterio-sclerosis), the myocardium, gastric mucosa, lungs and kidneys[1 9 11 19]. This condition occurs most commonly in grazing animals which ingest plants containing a compound with 1,25-dihydroxycholecalciferol-like activity. The syndrome has a variety of names, each characteristic of the country involved, but "enzootic calcinosis" is most commonly used. All grazing species of animals have been known to be affected, and the disease has been reported from many countries, including South Africa. Plants incriminated include *Solanum malacoxylon, Cestrum diurnum,* and *Trisetum flavescens.* Enzootic calcinosis is characterized by calcification of the blood vessels, and of other tissues to a varying extent. It has a chronic course which can stretch over many years. The initial symptoms are mass loss and disturbances in the animal's mobility. The post mortem lesions are all referable to calcification of fibro-elastic tissue in all organs[18].

Hypercalcaemia may occur when there is extensive neoplastic or infective involvement of the skeleton, as occurs in osteosarcomas, multiple myeloma and sarcoidosis[1 12]. Pseudohyperparathyroidism has been reported in the dog. This condition occurs when a tumour, most frequently a lymphosarcoma, secretes a parathyroid hormone-like compound. This endogenous substance acts on the intestines, bones and kidneys in the same manner as normal parathyroid hormone, resulting in a hypercalcaemia[9].

Hypocalcaemia occurs with starvation, acute pancreatitis, hypoparathyroidism, ketosis, milk fever, other metabolic disorders, and in alkalosis. The result of hypocalcaemia is tetany in non-ruminant animals and flaccid paresis to paralysis in sheep and cattle. Starvation causes a milk hypocalcaemia due to the decrease in the concentration of serum albumin. Any other cause of hypoalbuminaemia also causes a mild hypocalcaemia.

Acute pancreatitis is usually accompanied by hypocalcaemia. This is due to the release of trypsin and lipase into the peritoneal cavity, which damages the mesenteric fat cells, releasing fatty acids. The fatty acids combine with calcium and precipitate as insoluble salts in the pancreatic tissue and the peritoneal cavity[3 9].

Hypoparathyroidism occurs most commonly when all of the glands are inadvertently removed during thyroid gland surgery. Very rarely, the parathyroid glands may be destroyed by inflammation, auto-immunity or invasion by tumours[1].

During ketosis, there is an increased renal excretion of calcium, probably associated with the acidity of the urine. This may result in a mild hypocalcaemia, but the severity is insufficient to result in clinical symptoms[1].

Milk fever or parturient paresis of dairy cows is the most important syndrome in animals, associated with hypocalcaemia. There is a decrease in the total serum calcium concentration, but this is poorly correlated with the severity of clinical symptoms. The concentration of serum-ionized calcium is closely correlated with the onset and severity of the physical signs. The cause of hypocalcaemia in milk fever is an inadequate absorption of calcium from the gastro-intestinal tract, and poor mobilization from the skeleton, along with the massive secretion of calcium into colostrum and milk. The primary cause of the imbalance now appears to be a metabolic alkalosis, which decreases the solubility of intestinal calcium, causes resistance of the skeleton to the action of parathyroid hormone, and increases the

albumin-binding of serum calcium, so decreasing the concentration of ionized calcium in the serum. The pathogenesis of the hypocalcaemia in heavily pregnant and lactating ewes, lactation tetany in mares, eclampsia in bitches, transit tetany in cattle and horses and hypomagnesaemic tetany in cattle have not been elucidated[12].

Serum inorganic phosphorus concentration is influenced by the dietary content of both inorganic phosphorus and organic phosphates. The organic phosphates are hydrolysed in the gastro-intestinal tract and are then absorbed. Phosphorus is principally utilized in the body for bone mineralization. Inorganic phosphorus is present in the serum in the forms of HPO_4^{2-}, $H_2PO_4^-$ and a little PO_4^{3-}. Inorganic phosphate is excreted in the urine, faeces and milk. Serum inorganic phosphorus concentration is influenced by[1]:

(a) The age of the animal. The amount of inorganic phosphorus in the serum is highest in young, growing animals.
(b) The phosphorus content of the diet. This is usually in excess of bodily needs in the food of omnivores and carnivores, but may be deficient in pastures grazed by herbivores.
(c) Vitamin D_3 increases the intestinal absorption of phosphorus to a small extent. It does increase the renal excretion of phosphates.
(d) Parathyroid hormone, in the presence of vitamin D_3, causes mobilization of skeletal mineral, which includes both Ca^{++} and HPO_4^{2-}. Parathyroid hormone also decreases the renal tubular reabsorption of phosphate, and so increases phosphate excretion.
(e) The concentration of serum calcium influences the serum inorganic phosphorus level, directly through the Ca: PO_4 solubility product; and indirectly via its control of the release of parathyroid hormone.
(f) The functional state of the kidneys influences the serum inorganic phosphorus level, as the kidneys are the main route of excretion. In advanced renal disease, phosphate is retained.

Hyperphosphataemia occurs with kidney failure, hypoparathyroidism, and occasionally during fracture healing and hypervitaminosis D. Kidney failure or advanced kidney disease causes a hyperphosphataemia due to phosphate retention. The high serum inorganic phosphorus concentration causes a depression in the serum calcium concentration because the solubility product of calcium and phosphate is exceeded. In addition, the damaged kidneys are no longer able to activate vitamin D, and the resultant low amounts of active vitamin D_3 enhance the hypocalcaemia, because of poor intestinal absorption of calcium. The hypocalcaemia stimulates the parathyroid glands to secrete parathyroid hormone, with resultant demineralization of bone, particularly the mandible and maxillae. This results in the fibrous osteodystrophy of secondary renal hyperparathyroidism[1 11].

Hypoparathyroidism, which is rare, causes a hyperphosphataemia owing to excessive renal reabsorption of phosphate in the absence of parathyroid hormone. A mild physiologic increase in the serum inorganic phosphorus level occurs during fracture healing. This is of no pathologic importance. Hypervitaminosis D increases the intestinal absorption of phosphorus, and so can result in a mild hyperphosphataemia[1 12].

Hypophosphataemia is most commonly seen in cattle on phosphorus-deficient diets. Sheep and horses are not as susceptible to the condition as cattle. Typically, cattle show osteophagia and pica; decreases fertility; decreased growth rate in calves; rough hair coat, often with hypopigmentation; enlargement of the epiphyses, and osteomalacia, resulting in a stiff gait and sudden lameness. A common complication of the osteophagia (allotriophagia) which accompanies phosporus deficiency is the ingestion of toxins of *Clostridium botulinum* in decaying carcasses. This causes an ascending paralysis and death, but no pathological lesions[11 12 19].

Transient hypophosphataemia usually accompanies the hypocalcaemia in milk fever in cows. This is due to decreased absorption of phosphates associated with the reduced appetite and the intestinal stasis that occur with this condition. During milk fever, there is decreased mobilization of skeletal mineral, as a result of alkalosis-induced resistance to parathyroid hormone. In addition, there is a high output of phosphates into colostrum and milk[1].

Occasionally in cows, low phosphorus intake is associated with haemolysis. This occurs in lactating cows, causing postparturient haemoglobinuria. The pathogenesis is not understood, but affected animals respond to phosphate administration, and the condition can be prevented by supplementing lactating cows with phosphorus. The grazing of cruciferous crops such as rape and kale causes a phosphorus deficiency, which also results in an acute haemolytic crisis[11 12 19].

Hypovitaminosis D is extremely rare in domestic animals in southern Africa. As a result of inadequate calcium and phosphorus absorption and decreased bone matrix formation, low levels of vitamin D_3 result in hypophosphataemia, and rickets in growing animals, while osteomalacia occurs in adults[1 9].

Hyperparathyroidism and pseudohyperparathyroidism cause a low serum inorganic phosphorus concentration, owing to the increased renal excretion of phosphates, stimulated by the excessive amounts of parathyroid hormone[1 9].

Serum magnesium concentration is influenced by the rate of absorption of magnesium from the intestinal tract, mainly in the duodenum. This is unaffected by vitamin D, but is decreased by high levels of dietary potassium, nitrogen, phosphorus, calcium, fats and an alkaline pH, as well as by diarrhoea. Most of the body magnesium is present in the skeleton (70%), with the remainder principally being intracellularly. The magnesium concentration in erythrocytes is approximately twice the plasma level, except in cattle. About half of the serum magnesium is ionized and half is bound to albumin. Magnesium activates various enzymes, influences muscle contractility, and is essential for neuromuscular nerve transmission. Magnesium is excreted by the gastro-intestinal tract, the kidneys, and the mammary gland[1 12].

Hypermagnesaemia of a mild degree occurs during parturition in cows; in cows with typical milk-fever symptoms and hypocalcaemia; in chronic infections; and in oxalate poisoning[12].

Hypomagnesaemic tetany occurs in cattle, usually milking cows, grazing magnesium deficient pastures, such as rapidly growing spring grass, wheat pasture or

winter grazing (low energy intake); and in calves receiving magnesium-deficient whole or skimmed milk. Hypersensitivity occurs and progresses to tetanic convulsions, which rapidly result in death. Post-mortem lesions are either absent or are restricted to congestion of the viscera, and agonal haemorrhages in the heart, and on the serosae, associated with asphyxiation because of paralysis of the respiratory muscles[12 19].

Sodium, potassium and chloride are involved in numerous physiological processes, including:

(a) maintenance of a normal osmotic balance (Na^+ and Cl^-);
(b) maintenance of a normal water balance and distribution (Na^+);
(c) maintenance of a normal acid-base equilibrium (K^+); and
(d) maintenance of neuromuscular function (Na^+, Cl^- and K^+).

Normally, sodium, potassium and chloride are almost completely absorbed from the gastro-intestinal tract. Sodium and chloride are principally extracellular ions, while potassium is the major intracellular cation. These elements are excreted through the kidneys. Sodium and chloride are retained when the renin-angiotensin system stimulates mineralocorticoid (aldosterone) release. However, water is concurrently retained, so there is no rise in the serum concentrations. The serum potassium level is decreased by the action of aldosterone on the kidneys, and by insulin release (which increases the transport of potassium into cells)[1 12].

Hypernatraemia occurs in dehydration; and in hyperaldosteronism (Conn's Syndrome), which has not been reported in animals. Hyponatraemia occurs with prolonged vomiting and diarrhoea; in water intoxication; in hypoadrenocorticism (Addison's Disease); and in uncontrolled diabetes mellitus.

Hyperkalaemia occurs with renal insufficiency, particularly in severe nephritis with oliguria or anuria; with urethral obstruction such as in cats or sheep with urolithiasis; in any condition causing acidosis or cellular disruption, such as in shock, massive trauma, pyothorax, peritonitis or bronchopneumonia; and in hypoadrenocorticism (Addison's Disease). Hyperkalemia depresses cardiac function, causing bradycardia and a decrease in the cardiac output.

Hypokalaemia is seen in decreased food intake, such as occurs in anorexia or starvation; in persistent vomiting and/or diarrhoea; during the rare occurrence of alkalosis; and in diabetes mellitus with ketoacidosis, resulting from the vomiting and loss in the urine. Hypokalaemia also occurs with persistent insulin release, such as occurs with insulinomas, or prolonged glucose administration causing hyperglycaemia. In dogs, hyperadrenocorticism (Cushing's Disease) does not result in a lowered serum potassium level unless there is a primary adrenocortical adenoma or adenocarcinoma excreting excessive quantities of mineralocorticoid, in addition to increased amounts of glucocorticoid hormone. Hypokalaemia results in muscular weakness and depression.

Hyperchloraemia occurs in dehydration; with liver cirrhosis; with any condition that increases capillary permeability resulting in the movement of serum proteins into the tissues, as for example in congestive heart failure or feline infectious peritonitis; and in respiratory alkalosis with the hyperventilation causing a reduction in the partial pressure on carbon dioxide.

Hypochloraemia accompanies respiratory acidosis, such as occurs with pneumonia, emphysema, pulmonary oedema, pneumothorax or paralysis of the respiratory muscles (as in botulism and tick paralysis); and in metabolic alkalosis. Diuresis of any cause also results in a lowering of the serum chloride concentration, for example with chronic interstitial nephritis, diabetes mellitus and diuretic therapy[1 9 12].

Lactic and pyruvic acids are intermediates of glucose and glycogen metabolism which readily diffuse into the blood. They are the end products of glycolysis, the anaerobic phase of glucose catabolism. The blood concentrations of both these acids rise when glucose catabolism increases. The serum lactic acid concentration rises with anaerobic metabolism. Thus, increases are seen with exercise; in hypoxia, such as occurs in pulmonary insufficiency, heart failure, and in severe haemolysis, such as with babesiosis, owing to the anaemia; and in shock. Lactacidaemia also occurs in ruminants with the ingestion of large amounts of soluble carbohydrate, such as in grain overload. Lactate is metabolized in the liver, so raised concentrations occur with severe liver disease. Lactacidaemia causes a metabolic acidosis[12 20].

An increase in the blood pyruvate concentration occurs after exercise, after eating, in anorexia, with malabsorption and diarrhoea, in thiamine deficiency, in ketosis and in severe liver damage[12 20].

The hydrogen ion concentration or the acid-base balance of the body is influenced by the food intake, disturbances in metabolism, losses through the alimentary tract, disturbances of respiration, and kidney function. The regulation of the hydrogen ion concentration is performed by the lungs and the kidneys. An increase in the partial pressure (concentration) of carbon dioxide, or a decrease in the pH (acidosis), stimulates the chemoreceptors and the respiratory centre, resulting in an increase in the respiratory minute volume. More carbon dioxide is then expired in the air. Conversely, a decrease in the partial pressure of carbon dioxide, or an increase in the pH (alkalosis), has the opposite effect, resulting in a reduced respiratory rate and depth, with retention of carbon dioxide. The kidneys excrete acid by exchanging hydrogen ions (H^+) with the sodium of sodium bicarbonate ($Na^+HCO_3^-$), sodium phosphate ($Na_2^+ HPO_4^{2-}$), and sodium chloride (NaCl) in conjunction with ammonia (NH_3). All these processes involve carbonic anhydrase activity in the renal tubule cell[8].

Acidosis and acidaemia are the terms used when the plasma pH falls below the physiological normal of 7,4. Alkalosis and alkalaemia are the terms used when the plasma pH rises above normal. As carbonic acid is the most rapidly and readily controlled buffer system of the body, all disturbances in the acid-base balance can initially be related to the equation:

$$H^+ + HCO_3^- \longleftrightarrow H_2CO_3 \longleftrightarrow H_2O + CO_2$$

Respiratory acidosis occurs when carbon dioxide is retained because of decreased pulmonary function. A respiratory acidosis occurs with depression of the respiratory centres, as occurs during general anesthesia or comatose states. Respiratory acidosis also occurs when alveolar gaseous exchange or diffusion is decreased. This occurs in pneumonia, pulmonary oedema, emphy-

sema and when there is a space-occupying mass or fluid in the thoracic cavity. In all these conditions, the acidosis stimulates the respiratory centre and the chemoreceptors, resulting in an increase in the respiratory minute volume. In addition, the kidneys excrete acid in the urine. Both of these mechanisms tend to compensate for the acidosis, raising the plasma pH towards normal.

Metabolic acidosis occurs when there is an increased production of acid by the body tissues, when there is a decreased excretion of acid by the kidneys and when there is an abnormal loss of cation through the gastro-intestinal tract. Conditions which increase the acid production in the body include ketosis in cattle; pregnancy toxaemia in sheep; diabetes mellitus with ketoacidosis; grain overload, or ingestion of much food high in carbohydrate in ruminants; and hypoxia of tissues, with anaerobic metabolism occurring, as is commonly found in shock. Nephritis with oliguria and anuria is the usual cause of a decrease in the acid excretion from the body. Cation loss through the gastro-intestinal tract as occurs with diarrhoea, results in a metabolic acidosis in young animals: the metabolic acidosis that occurs with colibacillosis in calves is probably the factor responsible for death[8].

Acidosis causes a decrease in ventricular contractile force, leading to a decrease in the cardiac output and a reduction in tissue perfusion. As a result, anaerobic metabolism occurs or continues, and more lactic acid is produced. In addition, acidosis reduces the responsiveness of the heart and peripheral blood vessels to the action of catecholamines. The result of the acidosis and the lactacidaemia is impaired tissue function, decreased energy production, cell damage and eventually cell death[20].

Respiratory alkalosis occurs when excessive carbon dioxide is excreted by the lungs as a result of an abnormal increase in respiratory volume. Respiratory alkalosis is rare in animals, but it does occur with heat stress; hyperventilation during general anesthesia; and excessive stimulation of the respiratory centre by drugs, chemicals or trauma resulting in brain stem haemorrhage or oedema[8].

Metabolic alkalosis occurs when acid is lost from the stomach because of vomiting, in cats and dogs. The alkalosis persists if there is pyloric obstruction, otherwise a metabolic acidosis occurs when duodenal contents containing a large amount of bicarbonate are also vomited. The acidosis is exacerbated by the concurrent tissue damage which occurs as a result of the primary aetiology, vomiting and the hypovolaemia. A metabolic alkalosis is a consistent finding with abomasal torsion in cattle, owing sequestration of acid in the organ. Iatrogenic metabolic alkalosis occurs with the overzealous administration of bicarbonate, and from absorption of excessive amounts of cation from the gastro-intestinal tract when salts such as $MgSO_4$ and Na_2SO_4 are given orally. The former is particularly serious when intestinal stasis is present and is not corrected by therapy. In herbivores, a metabolic alkalosis is associated with kidney failure, because cation is not excreted. The most common cause of renal insufficiency in cattle and sheep are nephritis, pyelonephritis and urolithiasis[8 11 19].

Alkalosis may or may not be evident clinically. In moderate cases, muscular weakness, mental confusion and muscle tremors are seen. If the alkalosis is severe, tetanic spasms of the muscles and coma may occur, due to a decrease in the level of ionized calcium in the blood.

Mixed disturbances are common and complex. For example, pneumonia causes a respiratory acidosis, as a result of an increase in the plasma partial pressure of carbon dioxide and a metabolic acidosis because of tissue damage and anorexia[8].

KIDNEY FUNCTION TESTS

Serum non-protein nitrogen includes all the serum compounds which contain nitrogen, except for the proteins. This group includes urea, creatinine, creatine, uric acid, ammonia, amino acids and non-specific nitrogen. The non-protein nitrogen concentration in serum represents a balance between protein intake, anabolic utilization, protein catabolism in intermediary metabolism and excretion[1].

The blood urea nitrogen (BUN) is the principal end-product of protein catabolism. The liver is responsible for the formation of urea. Urea is present in solution in the blood stream and in all body fluids where it serves no known useful purpose. Excretion is almost exclusively by the kidneys. A small amount is excreted through the skin in animals that sweat, and some is degraded by intestinal bacteria. Urea, being a small molecule, is filtered by the glomeruli. Approximately 40–60 % of this filtered portion diffuses back into the blood through the renal tubules. The rate of diffusion from the renal tubules into the interstitial fluid and so to the blood depends on the rate of transit of the glomerular filtrate along the tubules: the faster the intra-tubular passage, the less urea diffuses back into the blood, *and vice versa*[1 7 12].

The blood level of urea nitrogen is influenced by:
(a) protein intake and absorption,
(b) amino acid utilization for body protein,
(c) amino acid utilization for energy,
(d) protein catabolism,
(e) liver function, and
(f) glomerular filtration rate.

The BUN concentration may increase slightly when protein intake and absorption are high, such as in animals on an all-meat diet. A rise is seen when amino acids are utilized for energy, so releasing their nitrogen molecules; and when protein catabolism increases, as occurs in any general catabolic state with mass loss. For example, during vigorous prolonged exercise, in starvation, in any pyrectic condition, with malignant tumours and with severe burns. When haemorrhage occurs into the small intestine, the blood protein is digested and then absorbed. Much of the absorbed amino acids, ammonia and nitrogen is converted to urea by the liver. The most significant rises in the BUN concentration occurs when the glomerular filtration rate is decreased. Reduced glomerular filtration rate occurs with a diminished renal blood flow (pre-renal azotaemia), resulting from a reduction in the number of functioning nephrons (renal azotaemia) or owing to a decrease in urine outflow (post-renal azotaemia)[7].

Mild to moderate increases in the concentration of BUN are caused by a decrease in renal blood flow, and hence in the glomerular filtration rate. Pre-renal azotaemia is caused by shock, dehydration from any cause, and congestive heart failure, particularly right-sided[1 21].

Renal pathology which results in an increase in the BUN concentration, includes any damage to the nephron, whether the lesion occurs in the glomeruli or the tubules or affects the blood supply. The BUN concentration does not become elevated until at least 75 % of the nephrons have been destroyed[7 9 12]. Nephritis, acute, subacute or chronic, primarily interstitial (eg leptospirosis or non-specific bacterial infections) or glomerular (eg autoimmune deposition or antibody-antigen complexes on the basement membrane as occurs in systemic lupus erythematosus and pyometra) are the most common causes. Usually, the predominant lesion involves connective tissue proliferation, namely chronic interstitial nephritis[11]. Toxic tubular damage, resulting in impaired tubular resorption also causes an increase in the BUN concentration. This occurs in chronic poisoning by mercury, arsenic, thallium or chlorinated hydrocarbons; in ethylene glycol toxicity; in oxalate ingestion, including such oxalic acid-containing plants as rumex, oxalis, *Beta vulgaris, Psilocaulen, Drosanthemum* and *Mesembryanthemum* species, rhubarb, *Opuntia,* and *Agave americana;* in long-standing hypoxia; in acute haemolytic crises, when bilirubinaemia occurs; and with renal oedema[1 11 19 21]. Glomerular damage *per se* does not cause a rise in the BUN level, but as the conditions such as glomerulitis, amyloidosis, vascular changes or antibody-antigen complex deposition on the basement membrane are usually progressive, the tubular function becomes impaired, and the interstitial blood flow is decreased. These later changes result in a constant rise in the concentration of BUN[1 11]. In the early stages of kidney damage, the BUN concentration changes little, even when there is a large decrease in renal function, owing to compensation and hyperplasia by undamaged nephrons. However, once 75 % of the nephrons become non-functional, small additional amounts of damage result in large increases in the BUN concentration[21].

Post-renal conditions resulting in an increase in the concentration of BUN are restricted to urinary tract obstruction and internal rupture. The most common causes are urolithiasis with urethral obstruction in male cats and wethers, with or without bladder rupture; prostatic hypertrophy, a frequent problem in old dogs and in wethers grazing clovers containing oestrogenic compounds; neoplasms that compress the urethra, for example leiomyomas of the neck of the bladder in dogs, or prostatic adenomas or adenocarcinomas; and traumatic bladder rupture[1 11].

In ruminants, the blood urea is constantly recycled into the rumen, where it is degraded by the bacterial flora. Hence, its concentration does not rise significantly in kidney dysfunction until very late in the disease[12 21].

A large increase in the BUN concentration indicates renal insufficiency and is usually associated with a variable degree of toxaemia. The retention of urea *per se* does not cause toxaemia, but the associated waste products that are concurrently retained cause the symptoms and lesions of uraemia. Of importance are the products of nucleic acid degradation, hyperkalaemia, hyperphosphataemia, hypocalcaemia, hyponatraemia, and the metabolic acidosis[11 12 21]. The lesions found most consistently in chronic uraemia are the kidney pathology and the tissue changes caused by the retention of protein breakdown products. These tissue changes include an ulcerative necrotic stomatitis, associated with fibrinoid necrosis of arterioles and abnormal saliva constituents; and hyperaemia and ulceration of the gastric and intestinal mucosa, owing to arteriolar necrosis and mucosal infarction. Renal retention of phosphates causes hypocalcaemia, which results in secondary renal hyperparathyroidism. This causes fibrous osteodystrophy and mineralization of the gastric mucosa, the respiratory system (alveolar ducts, arteriolar walls, and the parietal pleura) and/or the kidneys. Non-regenerative anaemia occurs in long-standing renal insufficiency owing to toxic depression of the bone marrow and/or deficiency in erythropoietin. The causes of pulmonary oedema and degenerative changes which sometimes occur in the liver are unknown. Terminal hyperkalaemia causes heart failure (the usual cause of death). The ventricles are hypertrophied and dilated, and the valves occasionally show nodular cicatrizations[11 19].

Decreases in the BUN concentration are seen when protein intake and absorption is very low, usually only in starvation; when anabolic steroids are given; and when liver function is impaired. Urea formation, from ammonia, by the hepatocytes is one of the last liver functions to fail. Hence, a decreased level of BUN only occurs with extensive hepatocellular damage, as for example in cirrhosis, neoplasia or when excessive portocaval shunting of blood by-passes the liver[1 9].

Serum creatinine is derived from creatine in skeletal muscle. A constant amount is released into the blood stream each day, dependent only on the animal's muscle mass. The serum creatine concentration is unaffected by age, sex, diet, exercise or catabolism. Creatinine is secreted into the intestinal tract where it is degraded by bacteria. The products of degradation, including ammonia and nitrogen are reabsorbed and metabolized by the liver to urea. Creatinine is excreted by the kidneys mainly by glomerular filtration, but also by tubular secretion to a small extent in dogs and cats. The serum creatinine conentration is principally dependent upon urine formation and elimination[1 7 12]. Serum creatinine concentration is only elevated when kidney function is seriously impaired. Rises in the serum creatinine concentration occurs with severe nephritis, severe toxic nephrosis, in terminal renal failure and with urinary tract obstruction. When renal disease is the cause, a raised creatinine concentration indicates a poor to hopeless prognosis[1 12].

Renal clearance tests measure the volume of plasma completely cleared of a substance in 1 min. Compounds which are excreted as a result of glomerular filtration only, indicate glomerular filtration rate; for example, inulin and creatinine (the small amount of tubular secretion of creatinine can be ignored for practical purposes). Compounds which are excreted both by glomerular filtration and by tubular secretion measure effective renal plasma flow and the kidneys' total ability to function; for example, para-amino hippuric acid (PAH) and phenosulphonphthalein (PSP). Other compounds are filtered by the glomeruli and are actively reabsorbed by the tubules, the amount of resorption depending on the serum concentration; for example, glucose. Renal clearance tests are performed by injecting a certain amount of the test compound intravenously, measuring its plasma/serum level at the beginning and at the end of the test period, and measuring the amount excreted in the urine during the test. The clearance per kg body mass is then calculated[21].

The clinical assessment of kidney function is most accurately based upon repeated urinalyses. The urine findings are the most informative of all the test results. The other tests, such as the BUN and creatinine concentrations, and the renal clearance tests, along with the microscopic examination of a kidney biopsy, are principally helpful in prognostication.

Additional tests which are performed in animal which are excreting very dilute urine (chronic interstitial nephritis; diabetes insipidus, partial or complete, hypophyseal or nephrogenic; psychogenic polydipsia; liver insufficiency; and hyperadrenocorticism) are a water deprivation test followed by the injection of vasopressin (Antidiuretic hormone), while the urine specific gravity, plasma osmolality and animal's body mass are monitored[21].

SERUM ENZYMES

The serum enzymes that are commonly assayed have no function in the blood but are derived from various body organs. The alteration in the concentration of an enzyme in the serum may indicate abnormal release of the enzyme from its parent cell, owing to an increase in the permeability of the cell membrane or to cellular death (necrosis); increased enzyme production; decreased enzyme production; or reduced excretion or degradation of the enzyme. Serum enzyme concentrations are used to indicate the functional status of the degree of damage suffered by an organ. Enzymes are removed from the serum either by excretion or by metabolic degradation or both. The diagnostic use of a particular serum enzyme concentration largely depends on the specificity of its distribution in the body[1 12].

Serum alkaline phosphatase (SAP) is widely distributed in the body, being mainly present in the osteoblasts, hepatocytes and biliary epithelial cells, intestinal mucosa, spleen, renal tubule cells, placenta and certain neoplasms. Alkaline phosphatase functions intracellularly in the liver, intestines, spleen and kidneys, to hydrolyze phosphoric esters with the liberation of inorganic phosphate. In the bone, alkaline phosphatase is produced by the osteoblasts but probably functions extracellularly. It is from the bone that the enzyme continually enters the blood, accounting for the majority of the amount found in serum in normal animals. In all animals, except the cat, SAP is excreted unchanged by the liver: cats excrete alkaline phosphatase in their urine. However, this does not appear to diminish rises in SAP concentrations significantly owing to pathological conditions in this species. Sheep and cattle have a very large normal range of SAP concentrations, so the diagnostic efficiency is much reduced in these species[1 2 6 9 10 12 16].

Raised concentrations of SAP occur in some categories of normal animals and accompanying liver and bone diseases. Young, growing animals or pregnant dams usually have moderately elevated SAP concentrations. The serum concentration of alkaline phosphatase rises dramatically in any liver condition that decreases the flow of bile, such as obstructive jaundice, bile stasis or hepatocellular damage and swelling (cloudy swelling or fatty infiltration) with obstruction of the intrahepatic bile canaliculi. Anoxia can result in these mild changes, with a large rise in the SAP concentration. Increases also occur in leptospirosis and hepatitis. The rise in SAP concentration may be due to a failure of excretion in the bile or to increased hepatocyte or bile epithelium production[1 2 6 9 10 12 16]. Dramatic rises in the SAP concentration are consistently found in hyperadrenocorticism (Cushing's disease), because of the induction of an isoenzyme not normally produced by the liver[5].

Bone diseases associated with raised SAP levels are characterized by an increase in the osteoblastic activity, for example rickets (very rare in South Africa), osteomalacia, panosteitis, osteogenic sarcoma (osteosarcoma), mixed mammary tumours, and in hyperparathyroidism of primary, secondary nutritional, or secondary renal origin. Occasionally, rises in the SAP concentration accompany diseases of the spleen, kidney and intestinal mucosa, particularly intestinal obstruction[1 2 6 9 10 12 16].

Serum alanine transaminase (SALT), formerly called serum glutamic pyruvic transaminase (SGPT), is present in large quantities in the hepatocyte cytoplasm of dogs, cats and primates and only in insignificant quantities in other cell types. It catalyzes the transfer of the α-amino group from the amino acid alanine to α-ketoglutaric acid. The enzyme is released when the liver cell membrane is disrupted. The low concentration found in the serum of normal animals is due to physiologic cell destruction. Rises in the SALT concentration occur when there is pathological destruction of hepatocytes, namely liver necrosis in dogs and cats. For example in hepatitis; intrahepatic cholestasis; severe babesiosis; severe anaemia due to anoxia; hepatic neoplasia, primary including hepatoma, and secondary; extensive lipidosis or fatty degernation of the liver, as occurs with hypothyroidism, hyperadrenocorticism or diabetes mellitus; leptospirosis; carbon tetrachloride or arsenic poisoning; suppurative hepatic necrosis; and toxaemias. The SALT level does not rise with chronic passive congestion of the liver, such as occurs in right heart failure. This enzyme is of no value for the diagnosis of liver disease in the horse, cow or sheep. Despite a half-life of only 2 to 4 days, the level of SALT remains elevated for approximately 7 to 8 days following hepatocyte injury, probably because of continuing cellular disruption as a result of the original insult[1 2 11 12 16].

Serum aspartate transaminase (SAST), formerly called serum glutamic oxaloacetic transaminase (SGOT), is present in the cytoplasm and mitochondria of many tissues in the body, in all mammalian species. It catalyses the transfer of an α-amino group from the amino acid aspartic acid to α-ketoglutaric acid. This enzyme is released into the serum when cellular membranes are damaged and remains elevated for approximately 2 weeks. Rises in the SAST concentration occur when there is extensive cellular disruption or necrosis of any tissue: it is non-specific. Increases are commonly found in association with muscle degeneration, as occurs in white muscle disease, azoturia or prolonged recumbency; myocardial damage; liver necrosis, as may be seen in chronic copper poisoning, aflotoxicosis or sporidesmin intake from *Pithomyces chartarum* (facial eczema or "geeldikkop" in sheep); and intestinal tract problems, such as colic or volvulus, and so on. Fascioliasis in cattle, liver fibrosis or cirrhosis do not cause rises in the level of SAST[1 2 6 10 11 12 16].

Lactic dehydrogenase (LDH) is present in the cytoplasm of all tissues of the body, in all mammalian

species. It catalyses the reversible oxidation of lactic acid to pyruvic acid. LDH is released when there is an alteration in the permeability of cell membranes or complete membrane disruption. Its level falls to normal within 24 h of cessation of organ damage. As a result of the ubiquitous occurrence of LDH, its release from erythrocytes with imperceptible haemolysis and the unexplained variability in the conditions that cause increases in the serum concentration, it has no rational use in the diagnosis of animal diseases[1 2 9 12].

The LDH present in the various tissues of the body vary slightly but significantly in chemical, physical and immunological characteristics. With advanced laboratory techniques (available in some large human clinical chemistry laboratories), these tissue-specific forms, or isoenzymes, of LDH can be determined. Unless isoenzyme analysis is performed, LDH assays are of historical interest only[2 9].

Sorbitol dehydrogenase (SDH) is primarily confined to the hepatocytes of the dog, horse, cow and sheep, where it is present in the ribosomes. It is released when there is severe, acute damage, as in necrosis. After cellular disruption has ceased, the serum concentration returns to normal within 7 to 9 d. SDH is virtually liver-specific in these species and is the enzyme of choice for the diagnosis of hepatic necrosis in horses and ruminants[2 6 12 15 17].

Glutamic dehydrogenase (GD or GLDH) occurs mainly in the ribosomes and mitochrondria of hepatocytes and in muscle to a lesser extent. The serum concentration returns to normal within 7 to 9 d after cellular disruption has ceased. It is used for the diagnosis of liver necrosis in cattle and horses[12 15].

Gamma glutamyl transpeptidase (γ-GT or GGT) occurs in the bile canaliculi epithelium and in the proximal convoluted tubule cells of the kidney. It is used to detect liver damage due to cholestasis and bile duct damage in the horse and cow. Its serum concentration does not rise in primary hepatocyte necrosis[15].

Creatinine phosphokinase (CPK) occurs in skeletal muscle, heart and neural tissue of all domestic animal species. The serum concentration becomes elevated in muscle damage such as is seen in white muscle disease, trauma, myositis, myopathy, thrombosis and convulsions; in myocardial damage such as myocarditis and endocarditis; or with severe muscular exertion. Serum CPK levels are moderately raised following severe exercise, parturition, surgery, administration of certain drugs such as halothane and adrenaline and the intramuscular injection of drugs. The serum level returns to normal within 2 to 3 d, and so only indicates current or active muscle damage[4 6 12].

Amylase is present in high concentrations in pancreas, salivary glands, intestinal mucosa and liver. It is excreted by the kidneys. Its serum level is used to diagnose acute pancreatitis. Hyperamylasaemia occurs within a few hours of the onset of acute pancreatic damage, and returns to normal within 2 to 8 d of the cessation of active damage. Other factors which may cause mild to moderate rises in the serum amylase level are stress; renal insufficiency; suppuration of the salivary glands; or the administration of corticosteroids or opiates (morphine or pethidine)[1 3 12 13 14].

Lipase is present in high concentrations in the pancreas. It is excreted by the kidneys. Serum lipase levels are used for the diagnosis of pancreatitis. Hyperlipasaemia occurs within a few hours of acute pancreatic damage, and usually remains elevated for 2 to 8 d. Elevated levels also occur with renal insufficiency, intestinal obstruction and after the administration of opiates[3 12 13 14].

LIVER FUNCTION TESTS

Serum bilirubin is derived from the degradation of haemoglobin in the reticulo-endothelial cells of the body. Bilirubin is transported to the liver bound to albumin, where it is conjugated with glucuronic acid. The conjugated bilirubin is then excreted in the bile. In the intestines, the conjugated bilirubin is metabolized by intestinal bacteria to many compounds, including urobilinogen. Some of the urobilinogen is absorbed in the colon. Urobilinogen is excreted by the liver in the bile and by the kidneys. Thus, 3 products resulting from the metabolism of haem are found in the blood:

(a) unconjugated or free bilirubin, which is measured as indirect reading bilirubin by the Van den Bergh test,
(b) conjugated bilirubin, which is measured as direct reading bilirubin by the Van den Bergh test, and
(c) urobilinogen.

Total bilirubin measures both unconjugated and the conjugated bilirubin present in the serum. Urobilinogen is only measured in the urine where normally a small amount is present. Either the absence or an increase in urine urobilinogen concentration is a significant abnormal finding. Conjugated bilirubin, which is soluble, may also occur in the urine in certain disease states, and in dogs normally, as canine kidneys can conjugate small amounts of bilirubin[10 21].

Table 1: BILIRUBIN LEVELS IN JAUNDICE

Initially prolonged	Normal animal	Haemolytic jaundice		Hepato-cellular jaundice	Obstructive jaundice	
		Initially	Prolonged		Initially	Prolonged
Total bilirubin in serum	N (low)	↑↑	↑↑	↑	↑↑	↑↑
Unconjugated bilirubin in serum	N (low)	↑↑	↑↑	↑	N	↑
Conjugated bilirubin in serum and urine	N (low)	N	↑	↑↑	↑↑	↑↑
Colour of faeces	Dark brown	Orange	Orange	Pale brown	Chalky cream	Chalky cream
Urobilinogen in urine	N (low)	↑↑	↑↑	↓	0	0

N, normal; ↑, increased; ↑↑, markedly increased; ↓, decreased; 0, absent

The total serum bilirubin and its 2 components are measured when icterus is suspected or diagnosed clinically, in order to determine the cause. The initial cause may be pre-hepatic or haemolytic, hepatic or hepatocellular damage, or post-hepatic or obstructive. However, as each of these conditions progresses, the effects of the initial pathology affect the function of the hepatocytes, and the picture becomes complex (Table 1)[1 2 10 16].

Pre-hepatic or haemolytic jaundice occurs when red blood-cell breakdown or haemolysis occurs at an excessive rate (Table 1). There is an increase in the concentration of unconjugated bilirubin in the blood. The hepatocytes conjugate this bilirubin and excrete it in the bile, causing the faeces to become an orange colour. An increased amount of urobilinogen is absorbed, and so the urine concentration of urobilinogen increases. However, if the haemolysis is severe or prolonged, hypoxia affects the liver-cell function and structure. Cloudy swelling, hydropic degeneration, and fatty infiltration of the hepatocytes occur. The liver's ability to conjugate bilirubin is one of the last functions to be lost, but the bile canaliculi are frequently blocked due to hepatocyte swelling. Thus the blood picture changes to one of an increase in both the unconjugated, and conjugated bilirubin levels. Raised levels of conjugated bilirubin are still excreted in the bile, so the faeces remain orange, and the urinary urobilinogen level remains elevated[2 10]. Horses are an exception: the majority of bilirubin in the serum following a haemolytic crisis remains unconjugated. Unconjugated bilirubinaemia also occurs with hepatocellular and obstructive jaundice in horses[2 6].

Conditions which cause a haemolytic crisis in animals include[2 10 11 19]:

(a) Dogs – babesiosis, haemobartonellosis/eperythrozoonosis, ehrlichiosis, leptospirosis, incompatible blood transfusions, autoimmune haemolytic anaemia, isoimmune haemolytic anaemia, snake bite, especially "boomslang" *(Dispholdius typus)*.
(b) Cats – babesiosis, haemobartonellosis, leptospirosis.
(c) Horses – babesiosis, equine infectious anaemia, neonatal isoimmune erythrolysis, phenothiazine poisoning.
(d) Cattle – babesiosis, anaplasmosis, *Eperythrozoon bovis*, acute leptospirosis in calves, bacillary haemoglobinuria (*Clostridium haemolyticum* = *Clostridium oedematiens* type D), *Clostridium welchii* type A, chronic copper poisoning, onion poisoning, snake bite, especially "boomslang" *(Dispholdius typus)*, potassium and sodium chlorate poisoning (weed killer), Cruciferae plant poisoning such as kale and rape, cold water haemoglobinuria, postparturient haemoglobinuria, isoimmune haemolytic anaemia (following previous anaplasmosis vaccination of the dam).
(e) Sheep and Goats – chronic copper poisoning, "enzootic icterus", eperythrozoonosis, *Acacia nilotica* poisoning (especially goats), Cruciferae plant poisoning.
(f) Pigs – eperythrozoonosis, acute leptospirosis in piglets, isoimmune haemolytic anaemia (following European Swine Fever/Hog Cholera vaccination of the sow).

Hepatic or hepatocellular jaundice occurs when the liver cells are damaged and swollen and block the bile canaliculi. The hepatocytes continue to conjugate bilirubin but it is not excreted and so regurgitates back into the blood. There may be some increase (usually mild) in the serum concentration of unconjugated bilirubin, due to a decreased uptake or conjugation ability on the part of the hepatocytes. However, the concentration of conjugated bilirubin is markedly raised. As less bilirubin is reaching the intestinal tract, the faeces become lighter brown in colour, and the level or urine urobilinogen decreases (Table 1).

Conditions which result in an hepatocellular jaundice in animals, includes[2 10 11 19]:

(a) Dogs – infectious canine hepatitis, leptospirosis, bacterial hepatitis, terminal cirrhosis, extensive neoplastic involvement of the liver.
(b) Cats – leptospirosis.
(c) Horses – Seneciosis, cardiac insufficiency, gangrenous pneumonia with toxic liver damage, infectious diseases, equine encephalomyelitis (Eastern and Western).
(d) Cattle – acute leptospirosis in calves, extensive liver abscessation due to traumatic reticulitis or rumenitis, nitrosamine poisoning, terminal Seneciosis, severe fascioliasis; poisoning with *Lantana camara, Lippia rehmani, Lasiospermum bipinatum*, or *Microcystis aeroginosa* (due to the phycocyan).
(e) Sheep – *Pithomyces chartarum* ingestion due to sporodesmin (*Tribulus terrestis*, "Geeldikkop"); poisoning with *Asaemia axcillaris, Lasiospermum bipinatum, Phomopsis leptostromiformis* (on Lupins), or *Microcystis aeroginosa* (phycocyan); congenital photosensitivity in Southdown sheep, severe fascioliasis.
(f) Pigs – acute leptospirosis in piglets, cresol or coaltar poisoning, hepatosis dietetica, gossypol poisoning (on cotton seed oilcake meal), iron poisoning in piglets.

Post-hepatic or obstructive jaundice occurs when there is an obstruction to the flow of bile either in the gall bladder or in the common bile duct. Initially, all the bilirubin is conjugated by the liver, and this is regurgitated back into the blood causing an increase in the concentration of conjugated bilirubin. As no bilirubin is reaching the intestinal tract, the faeces are a chalky white colour, and no urobilinogen occurs in the urine. As the condition progresses, the functional ability of the hepatocytes is impaired, and the liver's capacity to take up and conjugate the unconjugated bilirubin is decreased, so the serum concentration of unconjugated bilirubin rises (Table 1). Obstructive jaundice is rare in animals but may be seen with parasitic obstruction of the bile duct, particularly with Ascarid helminths, most commonly in the pig; bile calculi; tumours within the gall bladder or bile duct, or causing pressure from outside the biliary system; inflammation of the biliary tree; and, in the horse, starvation and constipation, resulting from intestinal tract stasis as there is no gall bladder in this species[2 10 11 15 19].

Bromsulphthalein dye (BSP) is an exogenous compound which is injected into an animal in order to determine the liver's ability to take up, conjugate and excrete substances. The method of performing the test

and the calculation of the result gives a figure which indicates the percentage retention of the dye in dogs but the excretion half-life of BSP in cattle and horses. The liver is the only organ which handles BSP, so this is a very specific test of liver function. The rate of uptake is dependent upon the blood flow to the liver, particularly the arterial flow, and so is reduced in dehydration, shock, congestive heart failure and liver cirrhosis. Bilirubin competes with BSP for liver uptake, so the test is difficult to interpret when hyperbilirubinaemia is present. The rate of uptake is independent of the liver's ability to conjugate the dye with glutathione: the conjugation step is rarely the rate-limiting process. Excretion of the BSP conjugate is via the biliary system, so any disease process that causes intrahepatic cholestasis, or posthepatic biliary obstruction decreases the rate of clearance from the blood. General liver pathology which has been associated with BSP retention includes hepatic lipidosis, such as occurs with endocrinopathies, or bovine ketosis, hepatic haemosiderosis; liver amyloidosis; centrilobular necrosis; periportal fibrosis; diffuse fibrosis; focal hepatitis; hepatic abscessation; infectious hepatitis, viral or bacterial; neoplastic involvement of the liver; secondary hepatic degeneration; heavy metal poisoning; and extensive fascioliasis. The BSP test is one of the most specific and sensitive tests available for the evaluation of liver integrity[1 2 10 15 16].

Serum uric acid is derived from purine and pyrimidine degradation (constituents of nucleic acids). The uric acid level is influenced by the level of ingestion and absorption of nuclear material, by the rate of cellular breakdown in the body and by the liver's ability to convert it to allantoin. Rises in serum uric acid level may occur in diffuse liver diseases, and in hepatocellular jaundice, but do not occur in obstructive or haemolytic jaundice[12]. In the Dalmation dog, only one third of the uric acid is converted to allantoin. Thus, the level of serum uric acid in this breed is higher than in the other canine breeds. The result of this metabolic difference is that uric acid occurs in the urine of Dalmations, and uric acid crystalluria may result[1 2].

Blood ammonia concentration is influenced by the intestinal absorption of ammonia (derived from bacterial breakdown of amino acids), its production as a result of protein breakdown in the body (deamination of amino acids by the liver), and its removal by the hepatocytes (which convert it to urea). Ammonia is a very toxic compound. Rises in the concentration of blood ammonia only occur in severe liver insufficiency or in portocaval anastomosis. Elevated blood ammonia concentration results in hepatic coma and neurological dysfunction[2 12 16]. A good example is *Crotalaria retusa* poisoning in horses, which causes "Walk-about Disease" or "Kimberley Horse Disease". The principal lesion is liver cirrhosis. This results in hyperammonaemia, with blindness and compulsive walking[11 19].

In conclusion, the numerous clinical chemistry tests available are extremely useful ancillary aids which help the clinician diagnose the organ(s) affected by a pathological process, indicate the nature of the damage and its extent and assist in prognostication. However, they must not replace clinical acumen and must always be considered in relation to the symptoms exhibited by the patient. The old motto: "When a diagnosis is not evident, examine the patient" is true, irrespective of the sophistication of the clinical laboratory. Unfortunately, that examination occasionally takes place in the post-mortem hall.

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BOOK REVIEW **BOEKRESENSIE**

PRINCIPLES OF VETERINARY RADIOGRAPHY

S.W. DOUGLAS & H.D. WILLIAMSON 3rd Ed.
Baillière Tindall, London 1980 pp. viii + 291 Figs. 363 Publ. Price R23.85

The main purpose of this book remains unchanged, namely "to provide students and practitioners with a concise practical introduction to the subject, which will enable them to equip and operate a radiographic unit suitable for the investigation of practically all routine veterinary clinical problems".

The first part of the book (106 pages) explains the theory of, and the equipment needed for, proper radiographic examination. The reader is supplied with essential information concerning the production of X-rays, the nature and the properties of the X-ray beam, estimation of exposure factors, recording of the image, darkroom layout, processing of films, the qualities of a diagnostic radiograph, and the dangers of exposure to radiation. The chapter on the dangers of radiation has been rewritten to bring it up to date with modern standards. The other chapters in this section were also updated in addition to some rearrangement of material.

The remaining part of the book (176 pages) consists of an atlas of positioning and is devoted mainly to the dog and horse. Each position used is explained and illustrated and useful exposure settings are provided to guide those who have no technique (exposure) charts for their X-ray machines. The radiograph obtained in each position is reproduced in miniature.

Illustrations of positions have been added to, or replaced, in a number of instances and the general quality of the reproductions of the radiographs improved in that their blackening now more closely approximates the blackening seen on good quality radiographs.

The use of positioning aids and cassette holders in the new illustrations is in accordance with modern requirements and underlines the importance of avoiding unnecessary radiation exposure, especially of the hands.

Some positions are included for the first time in this edition, namely an upright lateral view of the canine pharynx to show the normal relationship between the soft palate and the epiglottis; a more informative AP view of the canine frontal sinuses; a recumbent dorso-ventral view of the canine chest for demonstrating small quantities of pleural fluid; the 60 ° coronal pedal route for AP exposure of the equine navicular bone; and oblique views of the equine skull to outline the tips of the molar roots.

The new terminology recommended for describing direction of exposure is briefly outlined but has not been employed in this new edition. According to the authors "these terms have not gained widespread acceptance among veterinary radiologists, possibly because what may be gained in anatomical exactitude is lost in complexity".

The absolute correctness of two of the positions illustrated is debatable, namely:

(a) the flexed ventro-dorsal view of the hip joints (page 128) may be adequate for diagnosis of certain conditions but is not considered by some to be acceptable for the diagnosis of canine hip dysplasia or femoral neck fractures; according to them, the femurs should be flexed more so that they form cranial angles of about 45 ° with long axis of the body instead of being positioned at right angles to it;
(b) for proper isolation of the shoulder joint on a lateral exposure (page 110) the head and neck need to be pulled further dorsally to prevent superimposition of the cervical vertebrae.

The illustration, in this new edition, of the position necessary for a lateral view of the cervical vertebrae (page 165) is considered to be inferior to the one it is replacing, both from the safety and the illustrative points of view.

Although it is mentioned (page 165) that a flexed lateral view of the cervical spine may be required to demonstrate misalignment, it is unfortunately not illustrated. From experience we know that the degree of flexion applied for demonstration of cervical vertebral instability is often hopelessly inadequate, especially if the instability is located in the lower cervical area. Inclusion of an illustration showing the correct method of flexion would be appreciated in future editions.

A minor slip occurred on page 124 where the new illustration for lateral exposure of the metacarpus shows a non-screen film being used instead of a cassette, but the exposure factors supplied were not corrected.

A grid is an expensive item and it is surprising to see that the horse in the illustration on page 246 is allowed to stand on it. The piece of hardboard interposed above the grid will most certainly not prevent "cracking" of the grid. A small home-made tunnel with a thick layer of perspex on top to take the weight of the animal should be preferred.

The chapter on contrast medium techniques has also been brought up to date. Mention is made of the new compound Metrizamide which is recommended for myelography and a dosage rate is supplied, but unfortunately the dilution rate is not specified.

The passing of a stomach tube for administration of the larger volumes of Barium required for an adequate follow-through study of the gastro-intestinal system is certainly not as difficult as it is made out to be. It is decidedly superior to the giving of Barium per os as far as the results obtained are concerned and will hopefully receive more attention in future editions. The technique is routinely used by the reviewer, even in large breeds, without any sedation being necessary.

Passing of a stomach tube will also facilitate the introduction of air directly into the stomach for negative or double-contrast studies. This method of examination of the stomach has been neglected largely due to the difficulty or unreliability of feeding sodium bicarbonate preparations or carbonated beverages per os.

In the final chapter (17 pages) miscellaneous species like the ox, sheep, pig, cat, birds, small laboratory animals, reptiles, amphibia, fish and primates are very briefly dealt with and exposure guides provided. Most of the material in this chapter is included for the first time and in time will probably be elaborated upon. A suggestion for future editions is the inclusion of illustrations of, and advice on, the positioning of parrots and raptors.

A number of exposure charts, drawn up for 4 different portable X-ray machines, are provided in one of the two appendices. These charts certainly are valuable in that they give some idea of the exposures necessary for different regions. Ideally, however, the practitioner should be able to draw up a technique chart specifically for his own X-ray machine. Advice on the formulation of such a chart will further enhance the value of the book.

This excellent book is outstanding value for money and is the only one prescribed for the course in Radiography given to Veterinary Nurses at the Faculty. It can be recommended very strongly to veterinary students and, in view of the implications of the Hazardous Substances Act (Act 15 of 1973) which applies also to X-ray machines, to private practitioners who have X-ray facilities.

C.J. Roos

BOOK REVIEW **BOEKRESENSIE**

GUIDE TO MEAT INSPECTION IN THE TROPICS

J.R. MITCHELL 2nd Ed.
Commonwealth Agricultural Bureaux, Farnham Royal, U.K. pp vii + 95, Figs 30, Tabs 4, Publ. Price £6,00 (overseas)

Despite the broad-brush inference of the title, this little book is clearly intended for use in developing African countries, from which the author has evidently derived much of his meat inspection experience. The stated purpose is to serve as a quick reference for personnel engaged in meat inspection who already possess basic knowledge of anatomy, pathology, parasitology and bacteriology. Indeed, meat inspectors who have such knowledge but have not been taught about transport of slaughter stock, ante and post-mortem inspection, principles of abattoir construction and laboratory tests will derive benefit from the chapters on these subjects.

Unfortunately, the author has tried, within the space of less than 100 pages, to cover too many aspects of a wide-ranging subject varying from the primitive ("feeding condemned material to vultures . . . should be discouraged") to the sophisticated ("the maintenance of ambient temperature of 10 °C and internal meat temperature of 7 °C is very important") without any particular gradation. The result is a collection of pieces of information, some of which are sound, some incomplete, some cryptic and some highly arguable such as the view that total condemnation of carcasses affected with foot and mouth disease is not necessary, although recommended.

In short this book will undoubtedly contribute to the improvement of meat inspection where the present procedures are rudimentary, but it cannot be seen as a recommended addition to the reference books of a fully-fledged meat hygiene service in tropical or other regions.

J.T.R. Robinson

BOOK REVIEW **BOEKRESENSIE**

THE ARTHROPOD PARASITES OF VETERBRATES IN AFRICA SOUTH OF THE SAHARA VOLUME IV. PHTHIRAPTERA (INSECTA)

J.A. LEDGER
South African Institute for Medical Research, Johannesburg 1980 No. 56 pp v 327 Figs 261 Price not quoted

This long awaited volume on the lice of vertebrates in Africa south of the Sahara is much welcomed. At last a single book can be consulted, and by using the keys provided and assisted by line illustrations of most of the genera, lice can be identified to sub-order, family, genus and sometimes to species. Where a complete identification cannot be made, the literature which needs to be consulted is listed.

Perhaps the most valuable chapter in this book is the host-parasite list. Using this, it is possible, when identifying lice, to narrow the field considerably or at least to check the feasibility of initial identifications.

For the convenience of the practical worker, the lice of domestic animals have been listed and reviewed in a separate chapter. This reduces or eliminates the need for a search through the main taxonomic body of the book. The value of this chapter is further increased by a review of control methods against these lice.

Although this volume is essentially a critical review of the lice of the Subsaharan Region, there are also useful and interesting chapters on other aspects relating to lice. These include collection methods, microscopic preparations, curating and indexing collections; a discussion of the Ethiopean Zoogeographical Region, Ethiopean birds and mammals, and the evolution of the Pthiraptera. There is also an informative review of the biology of Phthiraptera under the headings diet, life-history and ecology.

This practical book should find wide acceptance amongst workers in the medical and veterinary fields as well as by biologists and students. For comparative purposes it will also be of great value to workers on lice outside Africa.

Dr. Ledger is to be congratulated on this well presented and comprehensive publication.

E.M. Nevill

FEATURE PAGE **TREFFERBLAD**

HYPERPLASTIC TOENAIL IN A CROSSBRED POODLE DOG

A 15-year-old male Poodle had been frequently presented for nail clipping over a period of 2 years, and it was observed that his medial toenail (digit 2) of the left foreleg was enlarging in size. When this nail became an encumbrence and impossible to cut with the conventional nail clippers, it was excised along with the distal phalanx (Fig. 1). Apart from the fact that the nail was very large (90 mm curved length), it was macroscopically normal in appearance (Fig. 2). No other toenails were affected in this way.

Microscopic sections were made and stained with H.E. These revealed hyperplasia of all the cellular layers of the nail, resulting in an appendage that seemed quite normal in shape and structure, although very large. The nuclei of the stratum basale and stratum spinosum were very large and vescicular (Fig. 3) with numerous nucleoli (arrowed). There was a sudden transition from skin (S) into keratinised tissue (K) (Fig. 4).

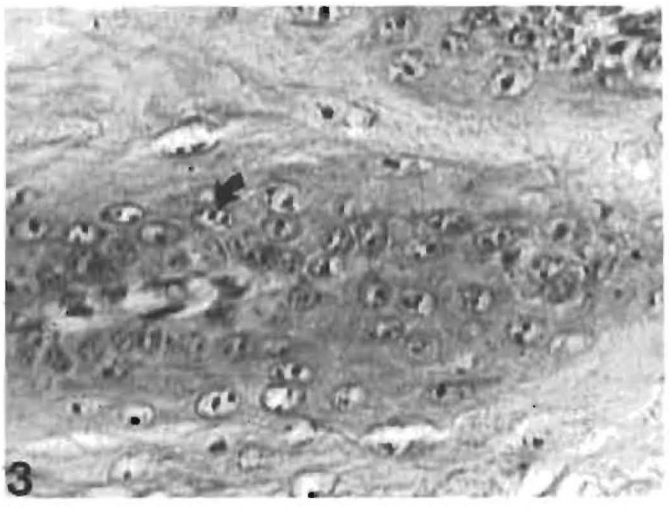

Fig. 1. Leg with digit 2 toenail excised.

Fig. 2. The hyperplastic toenail measured against a centimeter ruler.

Fig. 3. Stratum basale cells (H.E. X 350).

Fig. 4. Transition from skin (S) to keratinised tissue (K) (H.E. X 88).

HIPERPLASTIESE TOONNAEL BY 'N POEDELKRUIS HOND

Oor 'n tydperk van 2 jaar was dit nodig om van tyd tot tyd the toonnaels van 'n 15-jaar-oue Poedelkruis-hond te knip. Die mediale toonnael (digitus 2) van die linkervoorbeen het egter geleidelik vergroot. Die hond kon as gevolg hiervan baie moeilik loop, en uiteindelik het die nael so groot en dik geword dat dit onmoontlik was om dit met 'n naelknipper af te knip. Die distale falanks van die betrokke toon is toe geamputeer (Fig. 1). Behalwe dat dit baie groot was (90 mm geboë lengte), het die nael makroskopies normaal gelyk (Fig. 2). Al die ander naels was in alle opsigte normaal.

Mikroskopiese snitte is gemaak en gekleur met H.E. Daar was hiperplasie van alle sellulêre lae van die nael, en die gevolg hiervan was dat die nael se struktuur en fatsoen normaal was alhoewel besonder groot. Die kerne van die stratum basale en stratum spinosum was baie groot en vesikulêr (Fig. 3), met talryke nukleoli

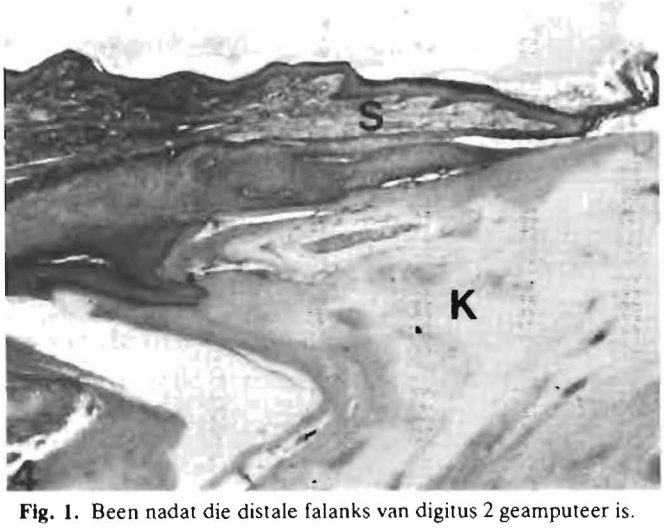

Fig. 1. Been nadat die distale falanks van digitus 2 geamputeer is.

Fig. 2. Die hiperplastiese toonnael by 'n sentimetermaatstok

Fig. 3. Selle van stratum basale (H.E. X 350).

Fig. 4. Oorgang van vel (S) na gekeratiniseerde weefsel (K) (H.E. X 88).

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There can seldom be an easily discernible difference between hyperplasia and neoplasia of structures. Since there was no invasion of multiplying cells into any specific areas, it may be assumed that this large toenail was formed by hyperplasia of the cell layers usually forming the nail. The aetiology is questionable, but may be related to trauma, which is responsible for the development of cornu cutaneum sometimes observed on the skins of animals after a particular area is traumatised. Lymphocyte infiltration into the basal cell layers of this nail could indicate chronic inflammation of those tissues due to continuous injury to that large structure.

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Thanks are extended to the lecturers of the Department of Pathology, Faculty of Veterinary Science, and especially to Prof R.C. Tustin for his interest shown, Prof W.H. Gerneke of the Department of Anatomy for his assistance and advice as well as to Mrs S.E. van der Hoven for the photography.

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(deur pyl aangedui). Daar was 'n skerp oorgang van die vel (S) na gekeratiniseerde weefsel (K) (Fig. 4).

Die onderskeid tussen hiperplasie en neoplasie van weefsel is nie altyd duidelik nie. Aangesien in hierdie geval daar geen prolifererende selle was wat enige spesifieke areas binnegedring het nie, kon aanvaar word dat hierdie groot toonnael ontstaan het uit hiperplasie van die selle wat normaalweg die nael vorm. Die etiologie hiervan is onseker. Dit mag die gevolg wees van trauma wat soms die cornu cutaneum veroorsaak op die velle van diere waar hulle aan trauma blootgestel is. Limfosietinfiltrasie in die basale sellae van hierdie nael mag dui op kroniese inflammasie van hierdie weefsel agv aanhoudende besering daarvan.

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