SA ISSN 0038-2809
Dewey Cat. No. 636.089

JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

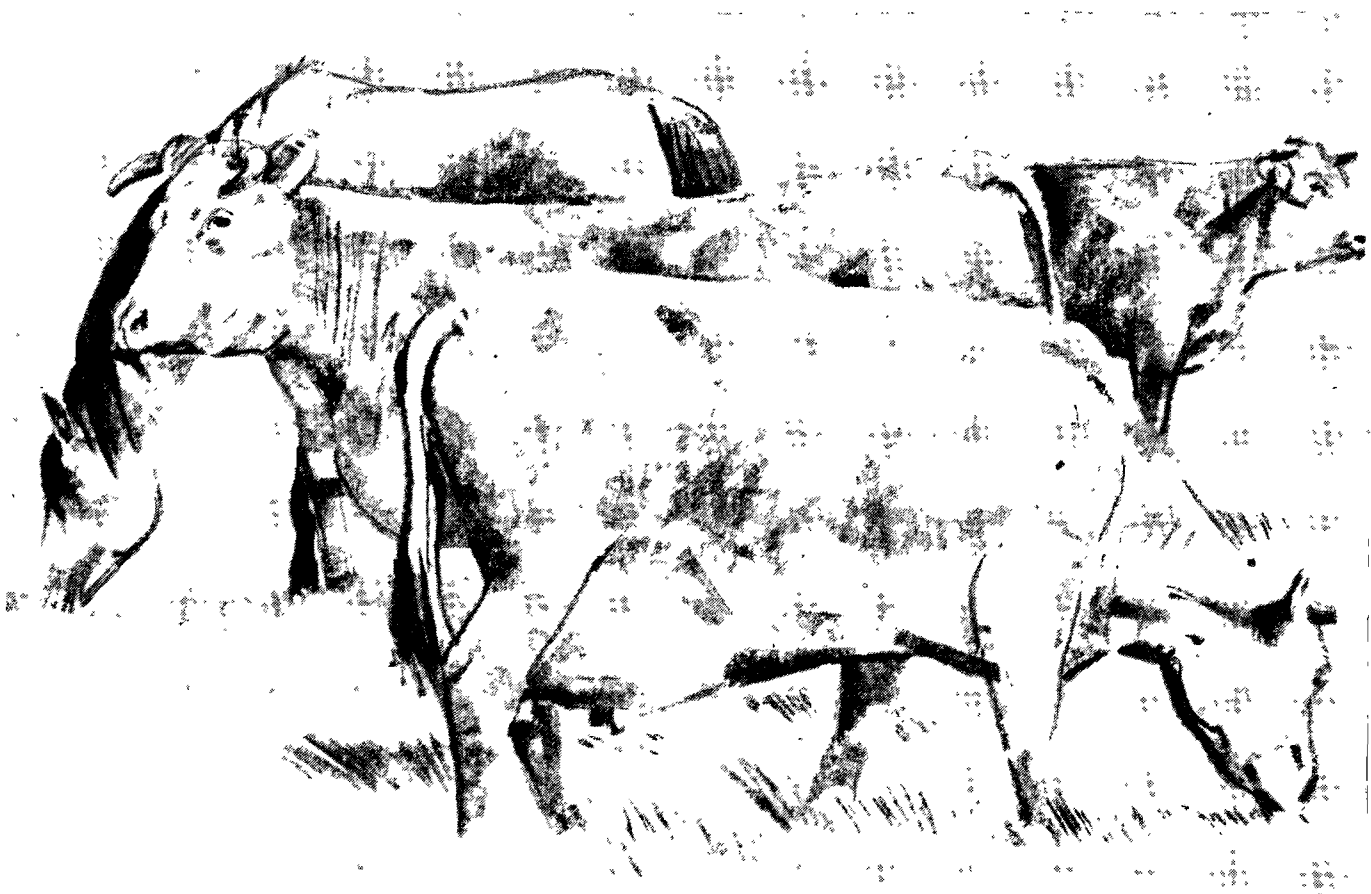
SEPTEMBER 1979
VOLUME 50 No. 3

SEPTEMBER 1979
JAARGANG 50 nr. 3

CONTENTS/INHOUD

Receptal®

(Synthetic releasing hormone for the release of both lutenising and follicle—stimulating hormones)



YOUR 12 POINT PLAN TO TREAT FOLLICULAR CYSTS

* RECEPTAL® contains the synthetic releasing hormone *Buserelin* This is equivalent to the natural LH/FSH releasing hormone in its chemical structure and in its action
* The only effect observed is the release of the gonadotrophin LH and FSH
* Thus RECEPTAL® has a physiological mode of action
* There is no risk of hormonal imbalance
* Receptal is synthetically produced and there is no variation in its biological activity
* Low dosage volume
* No adverse systemic or local effects have been observed following the use of RECEPTAL®
* No immunological response to repeated injected has been observed
* Manual rupture of cysts is unnecessary
* Ready to use solution for muscular injection
* No residue restriction period on the use of meat or milk
* RECEPTAL® has a short half-life of 5—10 minutes

Hoechst

Hoechst Pharmaceuticals (Pty) Ltd
3 Caxton Street Industria
P.O Box 8692 Johannesburg 2000

Veterinary Division

Index to Advertisers **Advertensie-Opgaaf**

JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

The JOURNAL is owned and published by the South African Veterinary Association, of which it is the official organ. It appears quarterly and is devoted to matters of veterinary importance generally. The statements made and opinions expressed by contributors are their responsibility only; such statements are not necessarily endorsed by the Editorial Committee, neither do the opinions reflect those of the Committee. The whole of the literary contents of this Journal is copyright.

SUBSCRIPTION. – A free copy of each issue is sent to all members of the Association in good standing. The subscription rate for local non-members is R12.50/a, post free; overseas subscriptions is R15,00/a, post free surface mail. BACK NUMBERS are obtainable at R4,00/number.

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 is 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 when submitting articles for publication. The senior author receives 25 reprints of each article free.

TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

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.

INTEKENING – 'n Eksemplaar van elke uitgawe word gratis aan alle volwaardige lede van die Vereniging gestuur. Die intekengeld vir plaaslike persone wat nie lede is nie, beloop R12,50/a, posvry; oorsese intekengeld is R15,00/a, posvry per land of seepos. VORIGE UITGAWES R4,00/eksemplaar.

BYDRAES – Die redaksie sal alle bydraes van veeartsenykundige belang vir publikasie oorweeg. Dubbelgespasieerde, noukeurig hersiende, getikte manuskripte moet in triplikaat (oorspronklike en twee afskrifte) ingedien word. Opset en verwysing moet die styl van hierdie uitgawe volg. MEER AS 20 VERWYSINGS word slegs met die goedkeuring van die Redakteur toegelaat. TABELLE en FIGURE moet in breedtes van 85 mm, of 176 mm, of in groottes van 263 × 176 mm weergegee word, of daartoe gereduseer kan word. Die getal figure en tabelle kan na oordeel van die redaksie beperk word tensy die outeur tot die koste van reproduksie bydra, veral kleurreproduksie.

Slegs die Internasionale Metrieke Stelsel (SI) word in hierdie Tydskrif gebruik, en outeurs moet sorg dat die korrekte SI eenhede vir vloeistofvolume, lengte, massa, tyd en stofhoeveelheid gebruik word. Tyd word uitgedruk as a (jare) week, d (dae), h (ure), min (minute) en s (sekondes). Verwys verder na M33a (SABS, P/sak X191, Pretoria).

HERDRUKKE moet ten tye van indiening van die bydrae bestel word. Senior outeurs kry 25 gratis.

ALL CORRESPONDENCE: Director, SAVA

Jl. S. Afr. vet. Ass., Box 26498, Arcadia 0007 (Tel. 26233)

ALLE BRIEFWISSELING: Direkteur, SAVV

Tydskr. S. Afr. vet. Ver., Bus 26498, Arcadia 0007 (Tel. 26233)

REDAKTEUR/EDITOR: PROF. H.P.A. DE BOOM.

REDAKSIE/EDITORIAL COMMITTEE:
A.J. DE VOS, P.G. HOWELL, A. IMMELMAN, R.K. REINECKE,
C.G. STEWART, R.C. TUSTIN, J. VAN NIEKERK, R.D. SYKES (Financial/Geldsake)
H.M. TERBLANCHE, L.W. VAN DEN HEEVER.

AGENTS IN GREAT BRITAIN: **AGENTE IN DIE VERENIGDE KONINKRYK**

Baillière, Tindall & Cassel, 8 Henrietta St.
Covent Garden, London.

ADVERTISING RATES on application **ADVERTEERTARIEWE op aansoek**

Financial subvention by the Department of National Education is gratefully acknowledged.

Geldelike steun deur die Departement Nasionale Onderwys word met dank erken.

COMMUNITY HEALTH AND THE PUBLIC HEALTH VETERINARIAN

1979 has officially been declared Health Year in the Republic of South Africa and the veterinary profession is vitally concerned with community health.

Veterinary Public Health has been defined as a component of public health activities devoted to the application of professional veterinary skills, knowledge and resources to the protection and improvement of human health. Veterinary public health activities involve a diverse range of functions within public health. These reflect the broad community of interests between veterinary and human medicine and indicate the opportunities for profitable interaction[1].

The veterinary profession is capable of and has a record of success in favourably influencing man's health in various ways –

(a) by bringing under control economically disruptive animal diseases as well as other costly but more insidious diseases of animals producing food and thus preventing malnutrition;
(b) by bringing under control diseases which are also zoonoses;
(c) by contributing to comparative and basic research which benefits human medicine;
(d) by participating in food protection and formulating many of the basic epidemiological concepts on which disease control in public health now rests;
(e) by promoting environmental hygiene; and
(f) by training of workers in the health field.

There is ample support and precedent in authoritative literature for the total involvement of the veterinarian in the promotion and maintenance of community health and for active veterinary participation in environmental health.

In South Africa veterinarians have a long and honourable history of contributing, both indirectly and directly, to the promotion of human health – indirectly by controlling and eradicating diseases of livestock which limit or prevent production of adequate supplies of food and fibre of animal origin; directly by dealing with diseases common to animals and man, i.e. zoonoses such as rabies and anthrax; more specifically, by controlling the hygiene of food of animal origin, particularly meat and milk, as part of the municipal health service.

The South African Veterinary Association and particularly the Veterinary Public Health Group is asking the question: "In what ways can the veterinary profession best serve the interest of human health in South Africa and what re-arrangements of policy and attitudes are necessary for such service to be rendered?"

In considering this question it is necessary to look at the present organisational structure in the Republic. There are two State Departments that are primarily involved:

(a) The Department of Agricultural-Technical Services in which the Division of Veterinary Services administers the Animal Diseases and Parasites Act No. 13 of 1956 and the Animal Slaughter, Meat and Animal Products Hygiene Act No. 87 of 1967.
(b) The Department of Health which previously functioned under the Public Health Act No. 36 of 1919, and now the Health Act No. 63 of 1977. It also administers various other Acts, i.e. the Foodstuffs, Cosmetics and Disinfectants Act No. 54 of 1972.

The Department of Agriculture Technical Services

Veterinarians have played a major part in the direction of municipal abattoirs and in the control of meat hygiene therein.

About 10 years ago the central control of meat hygiene in abattoirs was transferred from the Health Department to the Division of Veterinary Services in terms of Act 87. At the same time the Abattoir Commission was established and subsequently most large local authorities transferred their abattoirs to the Commission and its successor, the South African Abattoir Corporation. A meat hygiene service at these abattoris and at a number of new large private abattoirs is now rendered by the Veterinary Division. There are about 16 veterinarians engaged in full-time work connected with meat hygiene control in abattoirs.

The Department of Health

The Department of Health does not itself employ veterinarians although of necessity it must concern itself with the keeping of animals, food of animal origin, the zoonoses and environmental pollution by means of regulation (Health Act 63/1977), and must give direction to local authorities in the implementation of such legislation.

The Department relies on local authorities to implement health legislation within their areas of jurisdiction. It authorises and subsidizes local authorities to employ veterinarians to control aspects of milk and meat hygiene. However, the degree to which a local authority uses the services of a veterinarian is not specified.

At present some 12 veterinarians, representing less than 1,5 per cent of the total number of active veterinarians registered by the South African Veterinary Board, are engaged in the local authority public health service. The number of municipal positions open to members of the profession has not increased within the past five years. The work of municipal veterinarians is usually limited to aspects of meat and milk control, usually in the primary stages of production.

In the field of milk hygiene, full-time municipally employed veterinarians are involved in Durban (1), Cape Town (1), Pretoria (2), Johannesburg (2), and Port Elizabeth (1). They are responsible to the Medical Officer of Health in each case but the scope of their activities varies considerably. In some centres the veterinarian controls the milk from the cow to the consumer with the help of staff allocated to him for this purpose. In others the veterinarian supervises dairy cow health and diseases of the cow transmissible to man while other officials see to other aspects of milk hygiene. Only in Cape Town are municipal veterinarians still responsible for direction of the abattoir and for meat hygiene therein. The Peri Urban Areas Development Board of the Transvaal employs a single veterinarian to control and supervise both milk and meat hygiene. In some local authorities veterinarians control the hygiene of meat wholesale and

0038-2809/79/03/0147–0148

processing establishments and in others they are also involved in other aspects of food hygiene control.

The appointment of veterinarians and their duties and status within the local health department depends entirely upon the views of the Medical Officer of Health. There exists no uniformity between local authorities and no clear central direction as to the role of the veterinarian in the public health team. This situation may lead to strained relationships between the veterinarian and other health officials resulting in various degrees of dissatisfaction, distrust, jealousy and antagonism – emotions which do not favour the kind of cooperation which is essential to successful teamwork.

This situation results from the apparent absence of a definite policy and specific guide lines on the part of the State Health Department concerning the concept of veterinary public health. The decision of the Medical Officer of Health is based upon his own concept of how to run his Department and may well be influenced by pressure from other officials who resent the further advancement of veterinary activities into the public health sphere.

The veterinary profession is actively growing in numbers. Apart from immigrants to this country the number of new graduates will double from July 1980 to about 90 per annum. The chronic shortage of veterinarians experienced to date will hopefully soon be a thing of the past and new entrants into the profession will be critically examining all venues of employment. *Students are already asking searching questions about a career in veterinary public health. In honestly answering such questions, one cannot avoid the following conclusions:*

(a) The Health Act of 1977 makes no specific mention of the veterinarian's appointment or role in the public health sphere (despite the strongly worded comments submitted by the Association on the draft Health Bill)

(b) The only obvious avenue of employment in the public health field is the control of meat hygiene, as a State Veterinarian in the Department of Agricultural Technical Services, within the confines of the abattoir.

(c) There is no evidence of any positive or specific effort on the part of the Department of Health to encourage local authorities to utilise the services of the public health veterinarian.

(d) There appears little likelihood that the number of municipal veterinary posts will increase under the present system, the converse being more likely as abattoirs pass from municipal to Corporation ownership.

(e) There is no particular demand for the services of veterinarians in possession of formal post-graduate training in public health.

This is a rather unsatisfactory when taking into account that:

(i) Veterinary public health is an acknowledged discipline in all developed countries and recognised by international bodies such as the WHO/FAO of the United Nations as capable of making a significant contribution to man's welfare.

(ii) Basic under-graduate training of veterinarians in South Africa provides a detailed theoretical and practical course in veterinary food hygiene and public health* comparable to that in the European Common Market and other western countries. Such training meets the requirements of the South African Veterinary Board.

(iii) Provision is made for post-graduate training in public (M.Med.Vet.(Hyg.) and the post-graduate Diploma in Veterinary Public Health, some of it in collaboration with the latter and of the same standard as the DPH available to graduates. Yet of the 15 odd veterinarians who possess the DVPH only three are currently directly engaged in the State and municipal public health service.

(vi) Adequate veterinary manpower is likely to be available in the near future to meet all demands in the veterinary public health field in either a full-time or part-time capacity.

*144 h theory, 40 h laboratory practicals, 10 d abattoir practice and 5 d milk hygiene practice.

In examining possible developments in veterinary public health it is necessary to quote from an authoritative statement on the principal functions and fields of activity of public health veterinarians[1]:

"From his present and past roles in public health, it is clear that the public health veterinarian functions in at least three distinct contexts. The first and most obvious of these is a purely veterinary context defined by the many different relationships of lower animal and their diseases to human health and wellbeing. These activities reflect the unique qualifications of veterinarians and are usually the basis for the formation of veterinary public health units within health ministries and departments. The other two context are an essentially biomedical one and more holistic or generalist context. These include activities that veterinarians and other public health workers may be equally qualified to carry out. *Animal-related functions.* These functions of public health veterinarians include such things as responsibility for (1) the zoonoses, in particular for their diagnosis, surveillance, and control; (2) comparative studies on the epidemiology of non-infectious diseases of animals in which there may be environmental or other influences common to man and lower animals; (3) the interchange of information between veterinary medical research and human medical research and for application of the findings of veterinary research to human health needs; (4) determining the dangers to man of biting, toxic, venomous, and other hazardous or objectionable animals, and studying methods of controlling them; (5) the health aspects of the production, processing and marketing of foods of animal origin; (6) health related problems of other animal industries, including the safe disposal of animal wastes; (7) supervision of experimental animal colonies maintained by public health laboratory or research services; (8) provision of continuous working liaison between public health agencies and veterinary and other animal-related units in other branches of government, the veterinary profession, the animal-owning public, farmers' organizations, other agencies in agriculture, pet and other animal-related industries, and civic action groups such as humane societies, as well as (9) technical consultation on all other human health matters relating to animals and their diseases.

Biomedical functions. Although such purely veterinary functions as those listed above provide the basis for the establishment of veterinary units within the public health infrastructure, individual veterinarians are also qualified to perform many additional roles in public health by building upon their primary broad training in the basic biomedical sciences. These are all in fields of public health in which physicians and certain other members of the public health team also serve, such as: epidemiology in general; health laboratory services; production and control of biological products; protection of all foods; drug evaluation and control; general environmental health, including radiological health and environmental physiology; and most aspects of public health research, including research in reproductive physiology and fertility control. These types of activity by veterinarians may

take place within the public health infrastructure but are usually performed in another setting.

Generalist functions. Beyond these possible areas of responsibility in public health, which may reflect either his unique acquaintance with lower animals and their diseases, or his biomedical training and related qualifications, the public health veterinarian is one of several members of the public health team who may be qualified for general public health roles concerned with the administration, planning and co-ordination of public health programmes. This is so because of (1) the long and comprehensive programme of university education required of veterinarians, (2) broad overlap between veterinary medicine and human medicine with acquisition of common knowledge and skills and, most important, (3) the traditional emphasis in veterinary education upon preventive, economic, and population aspects of disease and health.

The relative priority that should be given to these components will depend partly on the level of socioeconomic development of the country, and partly on special factors that may operate in particular countries.

It is possible to make the broad generalization, however, that at first the emphasis should be on control of zoonoses and maintenance of basic levels of food protection. Subsequently, as socioeconomic development proceeds, these activities will become so well established that efforts can be extended to deal with problem areas more commonly associated with highly industrialized societies, such as environmental protection and comparative medical studies centred on noncommunicable diseases."

There is little presently available evidence of extension of the South African veterinarian's traditional restricted sphere of activities to ensure maximal utilisation of his training and capabilities and there is no indication of any increase in the scope and number of positions in veterinary public health available to the profession despite some degree of utilisation of available relevant post-graduate training facilities.

The veterinarian is undoubtedly entitled to a certain status and consideration by virtue of the fact that he is a professional man, registered with the S.A. Veterinary Board in terms of Act 16/1933 and capable of earning a good living by entering private practice or other spheres of veterinary work. He must, therfore be given the same kind of status as members of his sister professions i.e. medical and dental practitioners. By status is meant not only the financial considerations but also responsibility and authority. He must also be eligible for promotion to senior positions in both municipal and central government service. Unless he is given the necessary scope for utilising his training, ability and initiative within the public health sphere, he may well transfer to other positions where he can be assured of a more satisfying and rewarding career.

The Association sincerely believes that a clear and realistic statement of policy and intent by the Minister or Secretary for Health regaring veterinary public health is urgently needed.

Such a statement is essential in order that those responsible for veterinary training, i.e The Veterinary Board and the Faculty of Veterinary Science of the University of Pretoria, may take note and make whatever adjustments are indicated. In addition it is necessary that the profession should be fully aware of the position – whatever it may be, because only then can it assume whatever responsibilities are entrusted to it. Also, incumbents of veterinary public health positions in local authority service need to know where they stand and what prospects exist for professional and service advancement within their chosen field. Finally, Medical Officers of Health and other Health Officers need to know of the position so as to rearrange, where necessary, the situation within local health departments.

There appear to be three options open in regard to such a statement: Either

(a) "Veterinary public health services are not required within the framework of the South African public health scene;" or
(b) "The existing arrangements for employment of veterinarians in the public health field are adequate. No changes are therefore considered necessary;" or
(c) "The Public Health Service, both at central and local authority level, needs to utilise fully the training and abilities of veterinarians in furthering the aims and objects of community health services. In order to do so it is required that wherever economically and practically feasible, local or regional community health authorities are required to engage one or more veterinarians, preferably in possession of post-graduate qualifications in public health, to act in responsible positions in the promotion and maintenance of public health and in carrying out specific duties, as defined, on behalf of the Medical Officer of Health. In addition, a veterinary public health section will be established within the broad spectrum of veterinary public health and to advise the Secretary for Health on any matters which his training and abilities allow him to."

The existing demarcation between the areas of responsibility of the Departments of Health and Agricultural Technical Services concerning control of meat hygiene within and without the abattoir respectively clearly requires the creation of a Liaison Committee to promote co-operation and uniformity of approach. In addition, implementation of uniform legislation on a national scale is now necessary to replace local authority bylaws; central control of application of such regulations by local authorities will however be required. In regard to the siting of abattoirs, the Deparment of Health needs to be consulted. These considerations add considerable weight to the above motivation for the creation of a suitably staffed veterinary public health section within the State Department of Health.

REFERENCE

WHO 1975 Technical Report Series No 573 The Veterinary Contribution to Public Health Practice. Rept: Joint FAO/WHO Expert Committee on Veterinary Public Health, Geneva.

BIBLIOGRAPHY

Albertyn AAL 1965 Journal of the South African Veterinary Medical Association 36: 33–42
Boyd A R J 1961. Canadian Veterinary Journal 2(5)
Greathead M M 1963 Journal of the South African Veterinary Medical Association 34: 669–671
Horwitz B M 1963 Journal of the South African Veterinary Medical Association 34: 213–220
Kampelmacher E H 1975 Veterinary Record 97: 104–107
Louw A J 1962 Proceedings: Institute of Public Health p135–145
Matyas Z 1978 Bulletin of the World Health Organisation 56(5): 699–771
Meara P J 1976 Journal of the South African Veterinary Association 47: 105–111
Nelson H 1944 Journal of the South African Veterinary Medical Association 15: 125–000

O'Brien S V 1964 Journal of the South African Veterinary Medical Association 35: 93–96
Van den Heever L.W., Giesecke W H 1967 Journal of the South African Veterinary Medical Association 38: 107–114
Van den Heever L W, Giesecke W H 1972 Journal of the South African Veterinary Association 43: 259–262
WHO 1943 Technical Report Series No 83 World Health Organisation, Geneva
WHO 1956 Technical Report Series No 111
WHO 1962 Technical Report Series No 236
WHO 1964 Technical Report Series No 269
WHO 1966 Administrative patterns in veterinary public health. WHO Chronicle 20.
WHO 1973 Interrelationships between health programmes and socio-economic development. Public Health Papers No 49, World Health Organisation, Geneva
WHO 1974 Veterinary Public Health: a review of the WHO Programme – 2. WHO Chronicle 28: 178–189

BOOK REVIEW — BOEKRESENSIE

SURVEILLANCE FOR THE PREVENTION AND CONTROL OF HEALTH HAZARDS DUE TO ANTIBIOTIC-RESISTANT ENTEROBACTERIA. Technical Report Series No. 624 (ISBN 92 4 1206241)

REPORT OF A WHO MEETING

World Health Organisation, Geneva, 1978, pp 54. Sw.fr. 6. – French & Spanish editions in prep. Available in RSA from Van Schaik's Bookstore (Pty) Ltd., Box 724, Pretoria 0001.

The uncontrolled and excessive use of antibiotics in man and animals has led to a disturbing increase in drug resistance on the part of pathogenic organisms and is diminishing the effectiveness of life-saving drugs. There is therefore a need for more rational and coordinated administraion of antibiotics and for strict measures against their abuse.

In order to review the problems raised by antibiotic resistance among the enterobacteria and related organisms, a WHO Meeting on Surveillance for the Prevention and Control of Health Hazards due to Antibiotic-resistant Enterobacteria was held in October 1977.

This report outlines the factors involved in the emergence and spread of antibiotic resistance in enterobacteria, as well as the ways of controlling such resistance – mainly by means of surveillance. Appropriate laboratory methods and the collection and processing of data are described.

Among other recommendations, the group calls for the establishment of national and international surveillance programmes to monitor antibiotic resistance in enterobacteria. It recommends continuing education for medical practitioners, veterinarians and epidemiologists in the use of antibiotics in therapy and in feeds, as well as appropriate training for laboratory workers. It is recommended also that laboratories should use accurate and reproducible susceptibility tests on enteric bacteria so that the data collected are comparable. The group considers that clinical, microbiological and epidemiological data collected at the local, national, and regional levels should be analysed and made available to clinicians and health authorities for the formulation of national policies on the import, manufacture and use of antibiotics. The need for an information system for the collection and processing of data by computer was stressed.

The report lists areas requiring further research, including the development of simpler and more rapid susceptibility tests, methods for the analysis and interpretation of the results of such tests, studies of the clinical and epidemiological relevance of resistant bacteria in the environment and the development of effective methods for the control of resistant microorganisms of human and animal origin.

In view of the far-reaching public health significance of antibiotic-resistant bacteria in man, animals and the environment, this report is recommended reading for epidemiologists, clinicians, veterinarians, health administrators and public health laboratory workers who must all collaborate closely if surveillance programmes are to be effective.

L.W. v.d. H.

THE EFFECT OF THE DYE-MARKING OF MASTITIS REMEDIES ON THE INCIDENCE OF ANTIBIOTIC RESIDUES IN PRETORIA'S MARKET MILK SUPPLIES

B H BESTER and S H LOMBARD

ABSTRACT: Bester B H; Lombard S H. **The effect of the dye-marking of mastitis remedies on the incidence of antibiotic residues in Pretoria's market milk supplies.** *Journal South African Veterinary Association* (1979) **50** No. 3, 151–153, (En) Department of Dairy Technology, University of Pretoria, Pretoria 0002, Rep. of South Africa.

A 1973 survey on the incidence of inhibitory substances (mostly antiobiotic residues) in market milk supplied in Pretoria, on 3 195 herd milk samples, 65 tanker milk samples and 252 samples of pasteurised milk using the disc assay procedure with *Bacillus stearothermophilus* C 953 as test organism, revealed inhibitory substances equivalent to 0,005 IU penicillin/ml in 7,8 % of the herd samples, 29,2 % of the tanker samples and in 38,5 % of the samples of pasteurised milk. In 38,9 % of the positive herd milk samples and 73 % of the samples of pasteurised milk, penicillin was indentified with the aid of the penicillinase test. Some of the pasteurised milk contained inhibitory substances equivalent to more than 1,0 IU penicillin/ml; in some of the herd milk samples this figure exceeded 5,0 IU penicillin/ml.

A repeat survey was undertaken in 1977/78 to evaluate the effect of compulsory dye-marking of non-prescription mastitis remedies on the situation. In a total of 1 081 herd milk samples, 60 tanker milk samples and 112 samples of pasteurised milk, antibiotic residues were found in 2,13 % of the herd milk, 11,7 % of the tanker milk and 2,1 % of the pasteurised milk samples, with a much lower average concentration of antibiotic residues. The compulsory dye-marking of mastitis remedies had a beneficial effect on the occurrence of antibiotic residues in milk but did not ensure their complete absence, presumably because dye-marking was not made compulsory for scheduled preparations.

INTRODUCTION

In spite of reports which drew the attention to the high incidence of antibiotics in market milk in the Republic of South Africa[1,2] very little, if anything, was done to improve the situation. The market milk industry was either ignorant of the health hazards imposed by the presence of antibiotics in their milk or it did not care. However, when yoghurt was introduced to this country during the second half of the previous decade, the industry often experienced difficulty in making yoghurt of a satisfactory quality. An increasing number of complaints were received of yoghurt that would not set properly and further investigation showed that in most cases the problem was caused by inhibitory substances in the milk and that these were mostly antibiotic residues. The growth in the yoghurt market coincided with a gradual switch over to machine milking which apparently caused *inter alia* an increase in the incidence of mastitis and consequently an increase in the use of mastitis remedies.

At its Annual General Meeting during November 1970 the South African Society of Dairy Technology adopted a resolution requesting the Minister of Agriculture to introduce legislation for the compulsory dye-marking of all antibiotics intended for intramammary use. This request to the Minister was repeated the following year.

During 1973 a survey of the occurrence of antibiotic residues in market milk supplied in Pretoria was undertaken. These results were not published at the time but were presented to the Municipal Health Authorities, the Department of Health and the Department of Agricultural Technical Services.

In terms of the powers vested in him by Act 36/1947 the Registrar of Veterinary Medicines required that all mastitis remedies sold directly the the farmer without a prescription from a veterinary surgeon be coloured with certain food dyes as from 1st July 1976. In order to determine whether the compulsory dye-marking of mastitis remedies had an effect on the incidence of antibiotic residues in milk, another survey was undertaken during 1977/78. The results of this survey, as well as those of the 1973 survey, are presented in this paper.

MATERIALS AND HANDLING

Milk samples

Samples of the milk of individual dairy herds supplying milk to Pretoria were taken from the farm bulk tanks by inspectors of the Milk Board. The samples were transported in insulated containers and tested on the same or on the following day. In the latter case the samples were kept refrigerated until being tested.

Samples of tanker milk were taken at the local dairies by inspectors of the Milk Board.

Samples of pasteurized milk were taken from home deliveries in six different suburbs and at local dairies. Care was taken that all these samples carried different date codes or that samples bottled on the same day were sampled from different delivery routes.

All samples were heated at 82 °C for 5 min in order to eliminate naturally occurring heat-labile inhibitory substances[3].

The test method

The presence of inhibitory substances was determined by the disc assay procedure[4] using *Bacillus stearothermophilus* C 953 as test organism at an inoculum concentration of 10 % instead of the prescribed 20 %. All tests were read after 5 h incubation at 55 °C.

With each set of tests, discs saturated with milk containing 0,005, 0,05 and 0,5 IU (international units) of penicillin per milliliter, respectively, were included as controls.

If an inhibitory substance was present in a milk sample, its concentration was expressed as "penicillin equivalent" (PE), irrespective of whether the substance was in fact penicillin or not. The concentration was estimated from a standard curve which was constructed by using a series of different concentrations of penicillin in sterilised milk and plotting the diameters (in millimetres) of the inhibition zones against the logarithms of the penicillin concentrations (IU/ml), which is a linear function.

On account of the many variations in the test that are difficult to control on a day-to-day basis[2,5], the concen-

0038-2809/79/03/0151-0153

trations of inhibitory substances reported are merely estimates. Nevertheless, the use of controls with each set of tests helped to increase the accuracy of the estimates.

All samples found to contain inhibitory substances were retested to verify the results and in some cases an additional subsample was treated with penicillinase[4] and simultaneously tested in identical manner. At a later stage Bacto-Penase discs (Difco Laboratories) were used instead of penicillinase solution. The absence of inhibition by the penicillinase-treated sample (or around the penase disc) when the untreated portion of the same sample contained an inhibitory substance, was accepted as proof that the inhibitory substance was penicillin.

RESULTS

The results of the first survey conducted during the period January to September 1973, are summarised in Table 1.

A total of 3 195 herd samples was tested,of which 249 (7,8 %) contained inhibitory substances. Fifty four of the inhibitory samples were tested for the presence of penicillin by the penicillinase test and in 21 of these (i.e. 38,9 %) penicillin was identified.

The concentration of inhibitory substances in some of the herd milk samples was very high and in many instances the milk from one or two herds caused the milk in a whole tanker to be contaminated to the extent of more than 0,005 PE/ml and in some cases even more than 1,0 PE/ml. The tanker samples presented in Table 1 in most cases were taken from tankers on routes of *which the herds' milk, contained in that particular tank*er, were not tested on that particular day.

Of the 252 samples of pasteurized milk tested during this period (Table 1), 97 (38,5 %) contained inhibitory substances in excess of 0,005 PE/ml. As could be expected by virtue of the dilution effect when contaminated milk is mixed with uncontaminated milk, the concentrations of inhibitory substances found in the pasteurized milk were lower than those found in the herd and tanker milk samples. Thirty seven of the samples containing inhibitory substances were examined by the penicillinase test and in 27 of them (i.e. 73 %) penicillin was identified.

Table 1: THE INCIDENCE OF INHIBITORY SUBSTANCES IN MARKET MILK SOLD IN PRETORIA DURING THE PERIOD JANUARY TO SEPTEMBER 1973

Penicillin equivalent (IU/ml)	Pasteurized milk samples number positive	%	Tanker milk samples number positive	%	Herd milk samples number positive	%
0,005 to 0,009	24	9,5	6	9,2	37	1,2
0,010 to 0,049	46	18,2	6	9,2	95	3,0
0,050 to 0,099	14	5,6	2	3,1	39	1,2
0,100 to 0,199	5	2,0	1	1,5	28	0,9
0,200 to 0,499	6	2,4	1	1,5	24	0,8
0,500 to 0,990	0	0,0	1	1,5	14	0,4
1,000 to 4,990	2	0,8	1	1,5	9	0,3
5,000 and more	0	0,0	1	1,5	3	0,1
Total more than 0,005	97	38,5	19	29,2	249	7,8
Total samples tested	252		65		3 195	

The testing of milk of individual herds was terminated by the end of September 1973 but the testing of pasteurized milk was continued. The results of all the pasteurized milk tested from January 1973 to the end of June 1976 are presented in Table 2. These results include those presented in Table 1. During this period 569 samples were tested of which 169 (29,7 %) contained inhibitory substances in excess of 0,005 PE/ml.

After the dye-marking of mastitis remedies became compulsory (from July 1976), there was a sharp decrease in the incidence of inhibitory substances in pasteurised milk (Table 3). During the period July 1976 to November 1978, a total of 112 samples of pasteurised milk was tested. Only seven of these (6,25 %) contained inhibitory substances.

A survey of herd milk and tanker milk undertaken during the period August 1977 to October 1978 (Table 4) also showed a sharp decrease in the incidence of inhibitory substances as compared to the situation prior to the dye-marking of mastitis remedies. A total of 1 081 herd samples was tested, of which 2,13 % contained inhibitory substances (Table 4). This percentage is much lower than the 7,8 % found during the 1973 survey (Table 1). The concentrations of inhibitory substances found were also much lower. No samples contained more than 1,0 PE/ml, whereas 0,4 % of the herd samples tested during 1973 (Table 1) contained more than 1,0 EP/ml.

Table 2: THE INCIDENCE OF INHIBITORY SUBSTANCES IN PASTEURIZED MILK SOLD IN PRETORIA DURING THE PERIOD JANUARY 1973 TO JUNE 1976.

Penicillin equivalent (IU/ml)	Positive samples number	%
0,005 to 0,009	56	9,8
0,010 to 0,049	76	13,4
0,050 to 0,099	18	3,2
0,100 to 0,199	10	1,8
0,200 to 0,499	7	1,2
0,500 to 0,990	0	0,0
1,000 to 4,990	2	0,4
5,000 and more	0	0,0
Total	169	29,7
Total samples tested	569	

Table 3: THE INCIDENCE OF INHIBITORY SUBSTANCES IN PASTEURIZED MILK SOLD IN PRETORIA DURING THE PERIOD JULY 1976 TO NOVEMBER 1978

Penicillin equivalent (IU/ml)	Positive samples number	%
0,005 to 0,009	2	1,8
0,010 to 0,049	1	0,9
0,050 to 0,099	3	2,7
0,100 to 0,199	1	0,9
0,200 to 0,499	0	0,0
0,500 to 0,990	0	0,0
1,000 to 4,990	0	0,0
Total more than 0,005	7	6,25
Total tested	112	

Table 4: THE INCIDENCE OF INHIBITORY SUBSTANCES IN MARKET MILK SOLD IN PRETORIA DURING THE PERIOD AUGUST 1977 TO OCTOBER 1978

Penicillin equiv. (IU/ml)	Herd milks samples No. positive	%	Tanker milk samples No. positive	%
0,005 to 0,009	5	0,46	3	5,0
0,010 to 0,049	10	0,925	1	1,67
0,050 to 0,099	2	0,185	1	1,67
0,100 to 0,199	2	0,185	1	1,67
0,200 to 0,499	1	0,092	1	1,67
0,500 to 0,990	3	0,278	0	0
1,000 to 4,990	0	0	0	0
5,000 and more	0	0	0	0
Total	23	0	7	11,7
Total samples tested	1 081		60	

DISCUSSION

The growth of the test organism used in the surveys reported on in this paper, is inhibited particularly by penicillin and to a lesser and varying extent by other antibiotics and inhibitors[2 4]. Iodophors, quaternary ammonium compounds and chlorine-based disinfectants generally used in the dairy industry affect the disc assay procedure with *B. stearothermophilus* C 953 as test organism only at concentrations much higher than could be expected in milk[6]. One can, therefore, assume that the inhibitory substances found in the milk samples examined during the surveys reported on in this paper were antibiotics. This assumption is strengthened by the fact that penicillin was positively identified in approximately 39 % of the inhibitory herd milk and 73 % of the inhibitory pasteurised milk samples tested. These figures could be much higher if one considers the fact that synthetic penicillins, such as cloxacillin and methicillin, may not be inactivated by penicillinase under the conditions of the test and would therefore not be identified as penicillin[4 7].

The presence of antibiotic residues in milk can endanger the health of the consumers of the milk and also cause problems when such milk is used for the manufacture of cheese and other cultured milk products. From both a health and technological point of view, penicillin causes more problems than other antibiotics. Concentrations as low as 0,003 IU penicillin/ml can cause allergic reactions in extremely sensitive persons[8]. *S. thermophilus*, one of the two bacteria used in the manufacture of yoghurt, is affected by as little as 0,005 IU penicillin/ml[6] and is completely inhibited by 0,02 IU penicillin/ml[9]. Only results on milk samples containing inhibitory substances equavalent to at least 0,005 IU penicillin/ml, are reported in this paper. All these samples could therefore be expected to impair the manufacture of yoghurt and impose a hazard to public health.

The incidence and amounts of antibiotic residues found in market milk in Pretoria during the 1973 survey were alarmingly high. Quite a number of samples contained more than 1,0 IU penicillin equivalent/ml while a few contained even more than 5,0 IU penicillin equivalent/ml.

After the dye-marking of mastitis remedies became compulsory, there was a marked decrease in the percentage of milk samples containing antibiotic residues as well as a decrease in the amount of antibiotice whenever it was found to be present. The presence of antibiotic residues in milk, however, has not yet been completely eliminated.

A possible explanation for the fact that inhibitory substances (presumably antibiotics) could still be detected in the milk from time to time, is that a small amount of antibiotic can be excreted by untreated quarters of an udder of which one or two quarters have been injected with a dye-marked remedy, whereas the dye is not excreted by the untreated quarters. Another possibility is that an antibiotic not administered intramammarily but by other routes, can to some extent be excreted in the milk[10]. However, the main cause for antibiotics still being found occasionally in milk is presumably that remedies prescribed by a veterinary surgeon need not be dye-marked. In order to secure the maximum amount of success from the compulsory dye-marking of mastitis remedies, it should also be applied to those preparations used under the supervision of a veterinary surgeon.

ACKNOWLEDGEMENT

Acknowledgement is gratefully recorded to the management and personnel of the Milk Board in Pretoria who supplied the milk samples.

REFERENCES

1. Meara P J 1959 A survey of penicillin contamination of market milk supplies. South African Medical Journal 33: 821–822
2. du Toit J J 1965 [The disc assay with various isolates of *Bacillus stearothermophilus* as quantitative measurement of antibiotics in milk] (Afrikaans). M Sc Agric thesis, University of the Orange Free State, Bloemfontein
3. Hausler W J 1972 Standard Methods for the Examination of Dairy Products 13 th edn. American Public Health Association, Washington D C
4. I D F 1970 International Standard FIL-IDF 57. International Dairy Federation, Square Vergote 41, 1040 Brussels
5. Welch H C 1957 Problems of antibiotics in food as the Food and Drug Administration sees them. American Journal of Public Health 47: 701
6. Bester B H, Lombard S H 1974 [Tests for inhibitory substances in milk] (Afrikaans). South African Journal of Dairy Technology 6: 47–52
7. Borrie P F 1962 Antibiotics in milk. 1. Medical aspects. Journal of the Society of Dairy Technology 15: 15–17
8. Cuthbert W A 1968 Antibiotics. In; Chemical residues in milk. Annual Bulletin part V. International Dairy Federation, Square Vergote 41, 1040 Brussels
9. Wright R C 1962 Antibiotics in milk. 2. Dairying aspects. Journal of the Society of Dairy Technology 15: 17–21
10. Jepsen A 1962 Residues of disinfectants and antibiotics in milk, p 449–455. In: Milk Hygiene. WHO monograph series no. 48. World Health Organization, Geneva

RIFT VALLEY FEVER VACCINE – ANTIBODY AND IMMUNE RESPONSE IN CATTLE TO A LIVE AND AN INACTIVATED VACCINE

B.J.H. BARNARD

ABSTRACT: Barnard B J H **Rift Valley fever vaccine – antibody and immune response in cattle to a live and an inactivated vaccine.** *Journal of the South African Veterinary Association* (1979) **50** No. 3 155–157 (En). Veterinary Research Institute, 0110 Onderstepoort, Rep. of South Africa.

In a study of the response in cattle to a live and an inactivated Rift Valley fever (RVF) vaccine prepared from cell cultures infected with RVF virus, the effects of varying doses and combinations of these vaccines were compared. The antibody response to a primary injection of either vaccines was poor when measured by the serum virus neutralization test (SVN) and the haemagglutination-inhibition test (HI) but a booster dose of inactivated vaccine evoked a good anamnestic response in cattle previously injected with either of these vaccines. Cattle vaccinated with the live vaccine but negative to the SVN or the HI for RVF were immune when challenged with a virulent RVF virus isolated from a cow during 1974.

INTRODUCTION

As a result of the abortifacient properties of Smithburn's Rift Valley fever vaccine strain[6] and its low immunogenicity for cattle (Howell, 1972, personal communication) an inactivated RVF vaccine was developed at this institute[1]. During a subsequent outbreak of RVF, the inactivated vaccine was used on a large scale in cattle and pregnant sheep. The information obtained from State Veterinarians and farmers on the efficacy of the vaccine was satisfactory. However, further tests were considered advisable to determine the effect of different vaccination schedules and to compare the antibody response to both vaccines as well as the immune response to a live vaccine. This paper deals with these tests.

MATERIALS AND METHODS

Virus

Smithburn's neurotropic RVF strain[6] and a field strain of RVF virus previously described[1], were used for vaccine production, while another field strain isolated in 1974 and passaged 3 times in baby mice, was used for challenge of immunity.

Vaccines

The inactivated vaccines were prepared as previously described[1]. The live vaccine was prepared by seeding roller bottles of BHK21 cells with Smithburn's neurotropic strain[6]. The culture was harvested when approximately 75 % of the monolayers showed cytopathic changes, then diluted with phosphate buffer containing 10 % peptone and 5 % lactose (BLP), freeze dried and reconstituted to ensure that each animal received at least $10^{5.5}$ mouse lethal doses 50/ml -mouse LD50/ml).

The inactivated vaccine was prepared in a similar fashion, this time from the field strain of RVF-virus, harvested, and diluted with 9 parts of BLP. This suspension which had an infectivity titre of at least $10^{7.0}$ mouse LD50/ml was inactivated with formalin and mixed with an equal volume of Alhydrogel*.

*Danish Sulphuric and Phosphate Works Ltd.

Inoculation schedule

Twenty-five young Africander type cattle, susceptible to RVF and held under field conditions in a RVF free area, were randomly divided into 5 groups consisting of 5 cattle each, and injected according to the following schedule: Group 1 (Table 1) was injected with 10 ml of the live vaccine i.e. 10 times the normal dose of $10^{5.5}$ LD50/ml. Groups 2 and 3 were injected with 1 ml of the same vaccine and Groups 4 and 5 with 2 ml of the inactivated vaccine. After 3 months Groups 2 and 4 were injected with a booster dose of live vaccine while Groups 3 and 5 received a booster dose of inactivated vaccine.

Challenge

Five cattle vaccinated with the live vaccine and 3 susceptible control cattle were challenged 1 year post-vaccination. They were injected subcutaneously with 1 ml of a 10 % suspension of infective mouse brain in BLP containing 10^6LD and kept under observation for 2 weeks. Their temperatures were recorded and blood was collected daily for virus isolation in 1-d-old mice. Virus isolation was confirmed with the SVN test.

Serological tests

Blood was collected at various intervals before and after immunization. Using the sera so obtained, a previously described serum-virus neturalization test (SVN) was carried out according to the constant serum virus-dilution procedure. A haemagglutination inhibition test (HI)[2] at pH 6,1 was also performed in which a sucrose-acetone extract of RVF infected hamster liver was used as antigen.

RESULTS

Antibody response

The antibody response of each animal in the 5 groups is presented in Table 1.

The antibody response to the primary injection of both vaccines was very similar. Four out of the 15 cattle injected with the live vaccine (Groups 1, 2 and 3) developed HI antibodies and 3 developed SVN antibodies

0038-2809/79/03/0155–0157

Table 1: THE ANTIBODY RESPONSE OF CATTLE TO THE ADMINISTRATION OF A LIVE AND INACTIVATED RVF VACCINE

Group	Animal No.	Antibody response: Months after administration									
		1		3		4		7		12	
		HI	SVN	HI	SVN	HI	SVN	HI	SVN	HI	SVN
1	1	40	0	–	0						
	2	–		–	0						
	3	–	0	–	0	ND		ND		ND	
	4	–	0	–	0						
	5	160	1,2	10	0						
2	1	–	0	–	0	–	–	–	0	–	0
	2	40	2,1	20	2,1	160	2,0	160	1,5	40	0
	3	–	0	–	0	–	–	–	0	–	0
	4	–	0	–	0	–	–	–	0	–	
5	10	0	–	1,0	20	1,0	10	1,0	–	0	
3	1	–	0	–	0	640	–	–	0	–	0
	2	–	0	–	0	320	1,0	–	0	–	0
	3	–	0	–	0	1 280	2,5	160	>4,0	160	3,0
	4	–	1,2	40	1,7	5 120	3,8	1 280	>4,0	640	3,5
	5	–	0	–	0	20	–	–	0	–	0
4	1	–	0	–	0	–	–	–	0	–	0
	2	–	0	–	0	–	–	–	0	–	0
	3	–	0	–	0	–	–	–	0	–	0
	4	10	0	–	0	10	1,0	20	1,5	–	1,5
	5	20	1,8	10	0	40	1,2	20	1,0	–	1,0
5	1	–	1,0	–	0	80	1,2	40	1,0	10	1,1
	2	320	3,7	160	>4,0	5 120	>4,0	2 560	>4,0	2 560	>4,0
	3	–	0	–	0	1 280	1,8	160	2,0	80	2,2
	4	–	0	–	0	1 280	1,2	80	1,0	40	2,0
	5	–	0	–	0	1 280	1,0	10	1,0	–	1,0

HI = Haemagglutination inhibition titre
SVN = Serum-virus neutralization index
0* = <0,5
–** = <1:10
ND*** = Not done

against RVF within 30 d post-vaccination. There was no difference between the response in the cattle in Group 1 which were injected with 10 ml of vaccine and cattle in Groups 2 and 3 which were injected with 1 ml only. Four out of 10 cattle in Groups 4 and 5 injected with the inactivated vaccine developed SVN antibodies and 3 of them developed HI antibodies.

The antibody response to a booster dose of live vaccine was poor (Table 1; Groups 2 and 4) and only those cattle that responded to the primary injection responded to the booster dose with the production of both SVN and HI antibodies against RVF.

The booster dose of inactivated vaccine (Table 1; Groups 3 and 5) stimulated the antibody response significantly. Ten out of 10 cattle responded by deveoping antibodies and 8 of them by developing SVN antibodies as well. The best response was obtained in cattle in Group 5 which received the inactivated vaccine as both a primary and booster injection. The antibody levels obtained by cattle in this group were higher and persisted for a longer period (Table 1; Group 5).

Immunity

The immune response of 8 cattle challenged with virulent virus is presented in Table 2.

All the vaccinated cattle developed SVN antibodies after challenge irrespective of their antibody reaction on vaccination. However, they did not develop a viraemia or a temperature. In contrast the susceptible cattle showed a rise in temperature as well as a viraemia.

DISCUSSION

The antibody response to a primary injection of an inactivated vaccine depends to a greater extent on the antigenic potency of the injected antigen[4]. It has been shown that two vaccines of different antigenic potency, when used for primary immunization, evoked similar antibody titres but elicited markedly different anamnestic responses[5]. The pattern of response to living antigens is for the most part quite different from that observed with inactivated antigens. The response to a live vaccine is usually of life long duration in contrast to the short term immunity evoked by an inactivated vaccine.

In this study, however, very little difference was observed between the primary response to the 2 types of vaccine and increasing the dose of the live vaccine did not improve its antigenicity (Table 1, Group 1).

While a booster dose of live virus vaccine on the one hand may result in a somewhat lower percentage of takes and a less satisfactory antibody response, a booster dose of inactivated vaccine usually elicits a vigorous antibody response[4]. In this experiment the inactivated

Table 2: ANTIBODY AND IMMUNE RESPONSE OF CATTLE TO A LIVE RVF VACCINE AND CHALLENGE

	Antibody response after vaccination		Response after challenge			
Animal No.	SVN[1]	HI[2]	SVN	H	Temp.[3]	Viraemia
1	Negative	Negative	2,2	1:2560	38,0	Negative
2	Negative	Negative	2,8	1:320	38,2	Negative
3	Negative	Negative	1,0	1:10	38,2	Negative
4	Positive	Positive	1,8	1:2560	38,0	Negative
5	Positive	Positive	2,2	1:5120	37,9	Negative
6	Susceptible control		2,1	1:2560	40,2	Positive
7	Susceptible control		2,5	1:5120	40,1	Positive
8	Susceptible control		2,0	1:5120	40,4	Positive

1. SVN = Serum-virus neutralization index
2. HI = Haemagglutination inhibition titre
3. Temp. = Highest temperature recorded

RVF vaccine when given as a booster dose at 3 months significantly boosted the antibody response. The booster dose of live vaccine stimulated antibody formation only in those animals that reacted to the primary injection and the antibody levels were lower and of shorter duration than those elicited by a booster of the inactivated vaccine. These results are in accordance with the observation of low immunogenicity of Smithburn's strain[6] in cattle. In sheep, nothwithstanding considerable individual variation in antibody titre, it was shown that a live RVF vaccine in general produces a higher antibody titre than an inactivated vaccine[1]. In this work the opposite was true for cattle. The booster dose of inactivated vaccine stimulated the antibody response significantly in 10 out of 10 cattle whereas only 4 out of 10 animals reacted to a booster dose of live vaccine by the production of antibodies. The observation that the SVN test is insufficiently sensitive and/or that factors other than neutralizing antibodies play a role in the resistance against RVF infection[3] is confirmed by the result obtained in this work (Table 2). Even after 2 inoculations with Smithburn's neutrotropic RVF virus, only 2 out of 5 cattle developed detectable antibodies but when they were challenged they were immune. In addition all of them developed SVN and 4 developed HI antibodies. These results to a certain extent contradict a previous observation by Howell (personal communication) on the low immunogenicity of Smithburn's[6] RVF strain for cattle. Furthermore, it indicated that cattle can be immunized successfully against RVF infection with a live vaccine prepared from Smithburn's strain. Its abortifacient properties in sheep at least, however, must be borne in mind.

With more cattle reacting to the inactivated vaccine and with higher antibody titres obtained with it there is every reason to believe that the immunity evolked is sufficient to protect cattle against RVF for at least 1 y[2].

REFERENCES

1. Barnard B J H, Botha M J 1977 An inactivated Rift Valley fever vaccine. Journal of the South African Veterinary Medical Association 48: 45–48
2. Clarke D H & Casals J 1958 Technique for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. American Journal of Tropical Medicine and Hygiene 7: 561–571
3. Coackley W, Pini A, Gosden D 1967 The immunity induced in cattle and sheep by inoculation of neurotropic or pantropic Rift Valley fever viruses. Research in Veterinary Science 8: 406–409
4. Norrby E C 1967 First international conference on vaccines against viral and rickettsial diseases of man. Pan American Health Organization. Pan American Sanitary Bureau, Regional Office of the World Health Organization. 301–311
5. Norrby E, Lagercranitz R & Garod S 1965 Measles vaccination iv. Responses to two different types of preparations given as a fourth dose of vaccine. British Medical Journal 5438: 813–817
6. Smithburn K C 1949 Rift Valley fever. The neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine. British Journal of Experimental Pathology 30: 1–6.

AN OUTBREAK OF WHITE MUSCLE DISEASE IN LAMBS BORN OF EWES ON A ZERO GRAZING SYSTEM IN NATAL

R.W. BRYSON and G.F. ZUMPT

ABSTRACT: Bryson R.W.; Zumpt G.F.: **An outbreak of white muscle disease in lambs born of ewes on a zero grazing system in Natal.** *Journal of the South African Veterinary Medical Association,* (1979) **50** No. 3 159–160 (En). Regional Veterinary Laboratory, Allerton, Private Bag X9005, Pietermaritzburg 3200, Rep. of South Africa.

White muscle disease appeared in lambs born of ewes in the zero grazing group during the course of a comparative fertility trial on ewes on various systems of husbandry. Analysis of the feed showed barely adequate selenium in the maize silage and a deficiency in the soil on which the high lysine maize was grown. Treatment of the lambs with a selenium/vitamin E injection rapidly controlled the condition. This deficiency may be more widespread than is presently realised.

INTRODUCTION

White muscle disease has been reported from many countries, particularly New Zealand, where soils are notoriously deficient in selenium. Outbreaks have been recorded in the United States, Finland, Australia and the United Kingdom[1] and in South Africa[2]. As far as can be ascertained there are no published reports of this condition in Natal.

HISTORY

An outbreak of white muscle disease occurred at the Cedara Agricultural Research Station, Natal where various husbandry systems are on trial involving three experimental groups and one control group of pregnant ewes. This trial has been in operation for three years with no particular note being made previously of weakness in the lambs. The zero grazing group, consisting of 91 Merino ewes, of which 64 were mature and 27 younger (4 tooth and less), was the only one effected. Cases of white muscle disease in lambs of this group first appeared during 1978. Lambing in the 1978 season commenced in early August and was completed by the 4th September. The lambing percentage in this group had only been 60 % during the previous year. For the 1978 lambing season the lambing percentage was 125 (84 %) as compared to 101 (18 %) and 64 (37 %) respectively for the other two experimental groups. The weaning percentage, however, was significant: affected group: 54 (18 %) group 2: 75 (73 %) and group 3: 68 (97 %).

In the affected group 6 of the lambs born of the young ewes and 10 of the lambs of the older ewes had died. Most lambs were between the ages of 24 and 34 d when first noticed as being affected. Six affected lambs were sacrificed for diagnosis.

When diagnosis had been established, several animals affected at 24 d were treated with a parental injection of 1 m*l* a proprietary mixture of selenium and vitamin E*. This was given on the 25th and again on the 28th day of age and was followed by rapid recovery in the uncomplicated cases. The balance of the lambs was also treated, with no further mortality from this cause being recorded, although deaths from other causes did occur.

*Bio-Se V Injection, Burns (S.A. Cyanamid) containing selenium 1 mg and vit. E 68 iu/m*l*

FEEDING

As stated, the lambs affected all came from the zero-grazed group of ewes. Their daily feed ration per animal comprised:

Maize silage	3,5 kg/d
Ground lucerne hay	0,75 kg/d
Shelled yellow maize	0,25 kg/d
Eragrostis hay *ad lib.*	

In addition the young ewes were given 0,125 kg/d of carcass meal. A lick consisting of ⅓ salt and ⅔ bone meal was also available. From 3 weeks a creep feed was available to the lambs consisting of:

Maize meal	75 %
Ground lucerne hay	20 %
Carcass meal	3 %
Dicalcium phosphate	1 %
Sodium chloride	1 %

The maize silage, shelled maize, lucerne and Eragrostis hay were all grown at the Cedara Agricultural Research Station. High lysine maize seed was used for the first time. Ewes in the other groups were fed on similar rations but had access to kikuyu and rye grass for varying periods according to the trial protocols.

SYMPTOMS

First reports described the condition as "weakness" in the lambs in the affected group. They had become weak and were unable to stand or suckle. If supported, they were able to suckle weakly, or, if not, were bottle-fed on dam's milk. No treatment had been given and mortality was 100 %.

Our first examination showed the affected lambs to be 3–4 weeks of age and in reasonable condition, with no evidence of digestive disturbance and usually no pyrexia. Reaction to stimulation was present in all limbs. If assisted, lambs would stand up for a few seconds, general muscular tremors being noted. The muscles of hind legs felt hard on palpation. In three cases pneumonia developed, when pyrexia, dyspnoea, coughing and nasal discharge were observed. In these cases the intercostal and diaphragmatic muscles were involved. All these cases terminated fatally in spite of treatment with vitamin E/selenium injections and oxytetracycline.

0038-2809/79/03/0159–0160

NECROPSY FINDINGS

Post mortem examinations were carried out on 10 lambs, 6 of which had been sacrificed for diagnosis and 4 of which had died naturally from the disease. The most striking lesions were in the heart muscle. Plaque-like white areas of degeneration were visible under the endocardium throughout both ventricles and extended into the auricles in several cases. Skeletal muscle degeneration was easily seen and occurred mainly in the hind limbs. The affected areas were greyish white and bilaterally symmetrical. The obturator muscle was particularly affected.

Secondary pneumonia was seen in the three cases of which the respiratory muscles were involved.

Histopathological examination at the Veterinary Research Institute, Onderstepoort, confirmed this condition.

SOIL ANALYSIS

Samples of the soil on which the maize was grown were taken for selenium analysis. This was kindly carried out by CSIR Laboratory with the following results:

Soil from maize land	J1A	0,195 mg/kg
	J4A	0,130 mg/kg
Maize silage		0,33 mg/kg

DISCUSSION AND CONCLUSION

Diets contining less than 0,100 mg/kg of selenium are associated with the condition and soils with a selenium content of less than 0,50 mg/kg and forages with less than 0,1 mg/kg have been associated with the disease.

As stated, all maize feed used was grown on this soil and, although the silage selenium was adequate, this was fed in limited quantities only.

Vitamin E analysis was not feasible but it is possible that a concurrent deficiency of this may have been present as well.

There are many factors involved in the muscular dystrophy which occurs in this condition and the actual pathogenesis is not as yet fully understood. Treatment with vitamin E/selenium combinaions is empirical but effective and may be an essential adjunct to successful sheep rearing in Natal and possibly elsewhere. Further work is being undertaken at Cedara and this will be published in due course.

ACKNOWLEDGEMENTS

Permission from the Director of Veterinary Services to publish this paper and assistance from the staff at the sheep section of Cedara are acknowledged.

REFERENCES

1. Blood D C, Henderson J A 1968 Veterinary Medicine. pp 719–721. Ballière, Tindall & Cassell. London.
2. Tustin R C 1959 An outbreak of white muscle disease in lambs. Journal of the South African Veterinary Medical Associaion 30: 451–455.

BOOK REVIEW — BOEKRESENSIE

RESTRAINT AND HANDLING OF WILD AND DOMESTIC ANIMALS

MURRAY E. FOWLER

1st Ed., 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 is the first to cover all aspects and methods of restraint in domestic, laboratory and wild animals.

The text is divided into three parts – totalling 26 chapters, seven appendices and a good index.

The first part – General Concepts – covers the tools of restraint, rope work and chemical restraint with emphasis on stress, thermoregulation and medical problems of handling of animals. Particular attention is paid to an understanding of animal behaviour and its bearing on humane restraint.

Part two deals with domestic animals (including laboratory animals and domestic and waterbirds) each chapter under the subheadings of classification, danger potential, physical restraint, transport and chemical restraint.

Thirdly wild animals are discussed under the same subheadings with 11 chapters covering the main groups, e.g. small mammals, marine mammals, reptiles, etc.

Throughout, the text is concise and clear, well illustrated with photographs and drawings. Each chapter lists important references, should greater detail be required.

The tables are easily read but it is unfortunate that publication of this book coincided with the withdrawal of phencyclidine and the non availability of tilazol (CI-744) combination – both important and widely used restraint drugs.

This book is well recommended to both veterinary practitioner and student, being easily read and well indexed. It would also be useful to any person regularly handling animals.

L.P.C.

THE EFFICACY OF FENBENDAZOLE AT A DOSAGE RATE OF 7,5 mg/kg AGAINST NEMATODE INFESTATIONS IN CATTLE

F.S. MALAN

ABSTRACT: Malan, F.S. **The efficacy of fenbendazole at a dosage rate of 7,5 mg/kg against nematode infestation in cattle.** *Journal of the South African Veterinary Association* (1979) **50** No. 3, 161–163 (En) Hoechst Research Station, P.O. Box 124, Malelane 1320, Rep. of South Africa.

Fenbedazole, dosed to artificially infested cattle at 7,5 mg/kg live mass, was more than 80 % effective in more than 80 % of the treated animals against immature and adult *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp., *Bunostomum phlebotum* and *Oesophagostomum radiatum.*

INTRODUCTION

Fenbendazole: methyl 5-(phenyl-thio)-2-benzimidazole-carbamate (Loewe and Urbanietz[2]) is registered in the Republic of South Africa as an anthelmintic for cattle at a dosage rate of 10 mg/kg live mass.

The present paper reports the results of anthelmintic trials conducted according to the non-parametric method of Groeneveld & Reinecke[1]. Cattle were artificially infested with *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp., *Bunostomum phlebotomum* and *Oesophagostomum radiatum* and treated with fenbendazole at a dosage rate of 7,5 mg/kg live mass when the worms were in either the third larval stage, or the fourth larval stage, or adult.

MATERIALS AND METHODS

Sixty, three to five month old crossbred beef calves were housed in concrete-floored cattle pens. The calves were bought from farmers in the vicinity of the Research Farm at Malelane, Transvaal and were at that stage unweaned. Weaning took place in the pens. They were fed a calf concentrate twice daily and parasite-free lucerne hay was available *ad. lib.*

The animals were treated on two occasions with a broad-spectrum anthelmintic for nematodes and cestodes and were vaccinated against *Clostridium chauvoei,* pasteurellosis, chlamydiosis and botulism.

The calves were divided into three groups, each of which was infested orally on several occasions with the same numbers of infective larvae of *H. placei, O. ostertagi, Cooperia* spp. *(C. pectinata* and *C. punctata),* and *O. radiatum. B. phlebotomum* was infested percutaneously, by placing the infective larvae on the skin. These infestations were so planned that at the time of treatment the worms present would be in the third larval stage in the one group, the fourth in the next and fifth and adult in the third group. Fenbendazole at 7,5 mg/kg live mass was administered *per os* with a syringe.

Autopsies were conducted according to the methods described by Reinecke[3,4]. Worm recovery in all cases was achieved by washing the gastrointerstinal contents with a strong stream of water on a sieve with 150 μm apertures, and preserving the residues for subsequent microscopic examination.

In the treated animals worm counts were done by means of a stereoscopic microscope. In addition a 1/10 aliquot was drawn from each specimen, larvae present were separated from ingesta or digested gut wall, and examined with the aid of a standard microscope to identify larval stages.

In those control animals in which the total worm count of a particular species was estimated to exceed 1 000 worms, three 1/10 aliquots were examined by means of a stereoscopic microscope. If there were less worms total microscopic counts were done in the other controls.

Results were analysed by the nonparametric method[1] subsequently modified by Clark, cited by Reinecke[3]. There must be at least 5 controls and in the present series 7 animals were used. The median indicates the worm burden of the controls. Eleven animals are treated ($p< 0,10$) and the median of the controls is multiplied by 0,25 and only one of the 11 treated animals may exceed this figure to warrant a grading of Class A, i.e. 80 % effective in 80 % of treated calves. Simulation studies have shown that if there is a reduction in the control median of 75 % (median x 0,25) after treatment there is no chance that compounds which produce an 80 % reduction in worm burdens (or less) will be graded Class A.

RESULTS

The worm burdens of the two trials each with three groups of calves are summarised in Table 1.

H. placei

Third stage larvae: The median burden of the seven control calves was 209 with a variation between 87 and 1 160 worms. The range in worm recovery from the treated animals was from 0 to 2.

Fourth stage larvae: Worm recoveries from the controls varied from 39 to 1 504 with a median value of 668. Worm recovery from the treated animals was from 0 to 2.

Fifth stages and adults: The median burden of the controls was 256 with a variation between 61 and 389 worms. No worms were recovered from the treated animals.

On statistical analysis an A efficacy rating was obtained against each of the developmental stages.

O. ostertagi

Third stage larvae: The median burden of the seven control calves was 609 with a variation between 0 and 1 427 worms. The variation in worm recovery from the treated animals was from 0 to 85 worms.

Fourth stage larvae: Worm recoveries from the controls varied from 745 to 2 351 with a median value of

Table 1: WORM BURDENS OF CONTROLS AND TREATED CALVES ANALYSED BY THE MODIFIED NONPARAMETRIC METHOD[3]

	L_3		L_4		5 & A	
	Controls	Treated	Controls	Treated	Controls	Treated
	H. placei					
Range	87–1 160	0–2	39–1 504	0–2	61–389	0–0
Median	209	–	668	–	256	
X 0,25	52	0/11 > 52	167	0/11 > 167	64	0/11 > 64
Class	–	A	–	A	–	A
	O ostertagi					
Range	0–1 427	0–85	745–2 351	2–81	336–2 067	3–16
Median	609	–	1 262	–	1 175	–
X 0,25	152	0/11 > 152	315	0/11 > 315	294	0/11 > 294
Class	–	A	–	A	–	A
	B. phlebotomum					
Range	23–164	0–1	13–297	0–0	7–102	0–0
Median	82	–	27	–	37	–
X 0,25	21	0/11 > 21	7	0/11 > 7	9	0/11 > 9
Class	–	A	–	A	–	A
	Cooperia spp.					
Range	37–2 236	0–13	1 425–4 752	0–3	1 458–5 223	0–1
Median	1 076	–	2 312	–	3 315	–
X 0,25	269	0/11 > 269	578	0/11 > 578	829	0/11 > 829
Class	–	A	–	A	–	A
	O. radiatum					
Range	181–823	0–9	130–918	0–6	41–594	0–1
Median	383	–	540	–	373	–
X 0,25	96	0/11 > 96	135	0/11 > 135	93	0/11 > 93
Class	–	A	–	A	–	A

L_3 = third stage larvae; L_4 = fourth stage larvae; 5 & A = fifth stage and adults.

1 262. Worm recovery from the treated animals varied from 2 to 81.

Fifth stages and adults: The median burden of the controls was 1 175 with a variation between 336 and 2 067 worms. The burdens which were recovered from the treated animals ranged from 3 to 16 worms.

On statistical analysis an A efficiency rating was obtained against each of the developmental stages.

B. phlebotomum

Third stage largae: The median burden of the control calves was 82 with a variation between 23 and 164 worms. The highest worm count in the treated group was 1.

Fourth stage larvae: Worm recoveries from the controls varied from 13 to 297 with a median value of 27. No worms were recovered from the treated calves.

Fifth stages and adults: The median burden of the controls were 37 with a variation between 7 and 102 worms. Not a worm was found in any calf in the treated group.

On statistical analysis an A efficiency rating was obtained against each of the developmental stages.

Cooperia spp.

Third stage larvae: The median burden of the seven control calves was 1 076 with a variation between 37 and 2 236 worms. The variation in worm recovery from the treated animals was from 0 to 13 worms.

Fourth stage larvae: Worm recoveries from the controls varied from 1 425 to 4 752 with a median value of 2 312. Worm recovery from the treated animals ranged from 0 to 3 worms.

Fifth stages and adults: The median burden of the controls was 3 315 with a variation between 1 458 and 5 223 worms. The highest worm count in the treated animals was 1.

On statistical analysis an A efficiency rating was obtained against each of the developmental stages.

Oesophagostomum radiatum

Third stage larvae: The median burden of the seven control calves was 383 with a variation between 181 and 823 worms. The variation in worm recovery from the treated animals was from 0 to 9.

Fourth stage larvae: Worm recoveries from the controls varied from 130 to 918 with a median value of 540. Worm recovery from the treated animals ranged from 0 to 6.

Fifth stages and adults: The median burden of the control calves was 373 with a variation between 41 and 593 worms. Only one of the treated eleven calves had 1 worm, the rest had none.

On statistical analysis an A efficiency rating was obtained against each of the devleopmental stages.

DISCUSSION

Fenbendazole is registered in most European countries at a dosage rate of 7,5 mg/kg live mass for the treatment of parasitic nematodes of cattle. In the Republic of South Africa a dosage rate of 10 mg/kg live mass was registered for use in cattle and in these trials the dose was reduced to 7,5 mg/kg live mass.

There are a few observations of particular interest in this experiment. Firstly, the occurrence of *Cooperia*

spp. in the abomasum. Secondly, hypobiosis was not noticed in the development of nematodes. Thirdly, percentage infestation rates of larvae dosed to control calves were as follows:

Trial	*H. placei*	*O. ostertagi*	*Cooperia* spp.	*B. phlebotomum*	*O. radiatum*
L_3	8,8 %	17,8 %	19,5 %	2,9 %	17,9 %
L_4	14,0 %	36,7 %	48,1 %	2,6 %	21,3 %
A	4,6 %	29,3 %	57,6 %	1,5 %	15,2 %

L_3 = Third stage larvae trial
L_4 = Fourth stage larvae trial
A = Adult worm trial.

The possible reason for the lower infestation rates in the adult *H. placei* and *B. phlebotomum* trial is that the calves used were slightly older and in a better condition and therefore more resistant to infestation.

CONCLUSIONS

In the light of the above findings the anthelmintic efficacy of fenbendazole at 7,5 mg/kg live mass can be classified in terms of the requirements of the Registering Officer (Act 36 of 1947), as summarized in Table 2.

Table 2: EFFICACY CLAIMS IN CATTLE FOR FENBENDAZOLE DOSED AT 7,5 mg/kg LIVE MASS

Worm Species	3rd stage larvae	4th stage larvae	Adult worms
Haemonchus placei	A	A	A
Ostergagi ostertagi	A	A	A
Bunostomum phlebotomum	A	A	A
Cooperia spp. *(C. pectinata* and *C. punctata)*	A	A	A
Oesophagostomum radiatum	A	A	A

KEY

Class	Definition
A	More than 80 % effective in more than 80 % of the treated herd.
B	More than 60 % effective in more than 60 % of the treated herd.
C	More than 50 % effective in more than 50 % of the treated herd.
X	Ineffective

Most workers compare the mean worm burdens of treated calves with those of undosed controls. If this method of analysis is used, efficiency in these experiments varies from 95,1 % to 100 % (Table 3).

Table 3: MEAN REDUCTION OF WORM BURDENS OF CALVES TREATED WITH FENBENDAZOLE AT 7,5 mg/kg COMPARED WITH UNDOSED CONTROLS

Worm species	L_3	L_4	Adult
H. placei	99,9 %	99,9 %	100 %
O. ostertagi	95,1 %	95,7 %	99,7 %
B. phlebotomum	99,0 %	100 %	100 %
Cooperia spp.	99,5 %	99,9 %	99,9 %
O. radiatum	99,3 %	99,7 %	99,9 %

REFERENCES

1. Groeneveld H R, Reinecke R K 1969 A statistical method for comparing worm burdens in two groups of sheep. Onderstepoort Journal of Veterinary Research 36: 285 – XXX
2. Loewe H, Urbanietz J 1974 Fenbendazole a novel benzimidazole carbamate. Proceedings of the Third International Congress of Parasitology: 1396
3. Reinecke 1972 An anthelmintic test for the gastrointestinal nematodes of cattle. Onderstepoort Journal of Veterinary Research 36: 285
4. Reinecke 1973 The larval anthelmintic test in ruminants. Technical Communication, Department of Agricultural Technical Services. Republic of South Africa No 106

CANINE ENCEPHALITOZOONOSIS IN KENNELS AND THE ISOLATION OF *ENCEPHALITOZOON* IN TISSUE CULTURE

C.G STEWART*, A.F. VAN DELLEN† & W.S. BOTHA*

ABSTRACT: Stewart C.G., Van Dellen A.F., Botha W.S. **Canine encephalitozoonosis in kennels and the isolation of *Encephalitozoon* in tissue culture.** *Journal of the South African Veterinary Medical Association,* (1979) **50** No. 3 (En) 165–168 Dept. Infect. Dis., Fac. Vet. Science, University of Pretoria, Box 12580, Onderstepoort 0110, Rep. of South Africa.

The protozoan *Encephalitozoon* was isolated in primary kidney cultures from dogs originating from three separate outbreaks of encephalitozoonosis in kennels. The disease was characterized by a fading syndrome in young puppies with nervous signs developing in some cases. It was not possible to reproduce the clinical disease with cultured organisms in either normal dogs or dogs immunosuppressed with methylprednisolone. The organisms were, however, reisolated in primary culture from two immunosuppressed dogs but not from other experimentally infected dogs. The freezing of *Encephalitozoon* organisms in liquid nitrogen is described.

INTRODUCTION

Encephalitozoonosis has been reported from many mammals including man, dogs, cats, foxes and laboratory animals[15]. Canine encephalitozoonosis has been reported from East Africa[11], Rhodesia[6], South Africa[1,2], England[12] and the United States of America[14]. In South Africa the disease has also been reported in a cat[19], and laboratory rabbits and mice[5] and a microsporidian parasite was described as an incidental finding in adenocarcinoma cells from a human patient[6].

Encephalitozoon cuniculi has been previously isolated and propagaged in tissue cultures from a variety of host animals. Monolayer cultures of rabbit choroid plexus cells were used to isolate the organism from rabbits, hamsters and mice[13]. Suspension cultures of mouse lymphosarcoma cells were able to support the growth of a "mouse ascites agent" which was probably *Encephalitozoon*[9]. *A number of cell lines can support the growth of E. cuniculi*[3,8,18] including canine kidney, feline lung, bovine kidney, rabbit kidney[18], mouse embryo[3], canine embryo and hamster glial cell[8].

HISTORY

The owner of *Kennel A* was a breeder of Toy Pomeranians with a total of approximately 30 breeding females. The only other dogs on the property were approximately 8 Rhodesian Ridgebacks. Rabbits had been housed in the kennels immediatly prior to their use for dogs.

This kennel had a history of severe losses of pomeranian puppies between 4–8 weeks of age. The main clinical signs described by the owner were a fading syndrome with the puppies becoming progressively weaker until death. Nervous signs were also described in some instances but were less apparent. The use of an infrared lamp to keep puppies warm at birth and during the nursing period had reduced the mortality rate.

Dogs were housed in groups and each kennel had a concrete floor with a fenced in exercise yard which was not concreted but was maintained in a clean condition.

Encephalitozoonosis was confirmed histopathologically in two puppies from the same litter[2]. This was the first litter produced by this bitch but the grandmother had a history of producing puppies with nervous signs which had been diagnosed as "distemper". Subsequently the grandmother produced a puppy showing signs of hyperaesthesia, clonic spasms, chorea-like nervous signs and general incoordination. This case was recorded in a separate paper as case A[2]. The puppy was euthanazed and primary cultures prepared from one of the kidneys (DK 2).

The owner of *Kennel B* kept approximately 50 breeding bitches of many different breeds. The hygienic conditions under which these dogs were housed were very poor. The kennels were mostly cold, wet and dirty. The concrete floors were old and cracked. There was a communal exercising yard where all dogs were allowed to exercise together for several hours every day.

A 5-week old puppy was submitted for examination. It was in poor condition and was continually crying. There was a history of other puppies dying at about the same age. Because of this the animal was euthanazed and a kidney submitted for culture (DK 1).

The owners of *Kennel C* were breeders of Toy Pomeranians and Maltese poodles. There were over 100 breeding bitches on the property of which approximately 70 % were Toy Pomeranians. The owner had complained about the loss of puppies, usually between 4–8 weeks of age. No clinical cases were described after this age. The disease had started after the introduction of 60 breeding dogs and bitches from an outside source. Previous to this very few dogs had been brought in from outside and the kennel had been established for approximately 30 years. The main clinical signs described by the owner were a fading syndrome where puppies just became weak and died. Whole litters were lost with these signs. Nervous signs were also seen in some puppies. The puppies which first showed nervous signs were from the imported bitches and the owners thought that the introducion of these dogs had introduced the disease into their kennels.

The disease then spread throughout the kennel and appeared to be restricted to the Toy Pomeranians. At first it was diagnosed as distemper but in spite of regular vaccination and revaccination of all dogs, the disease persisted. In the previous 3 months to our visit 10 puppies had died out of 140 puppies produced and it appeared as if the incidence of the disease was on the

*Faculty of Veterinary Science, University of Pretoria.
†Major, USAD, V.C. Geographic Zoonoses Division, Armed Forces Institute of Pathology, Washington D.C. 20306. Temporary assignment: Department of Pathology, Veterinary Research Institute, Onderstepoort. The views expressed herein are those of the authors and are not to be construed as official or as reflecting the views of the U.S. Air Force of the Department of Defence.

0038-2809/79/03/0165–0168

decline as the losses had been higher than this in the past.

The hygienic conditions were of a high standard. All dogs were housed in kennels with outside runs which had concrete floors throughout. Groups of 10–20 dogs were kept in each kennel and only pregnant bitches were housed separately.

A puppy which had failed to thrive and had eventually died was submitted for routine post-mortem examination. Encephalitozoonosis was diagnosed. Subsequently a 7 week old litter mate of this puppy was submitted for examination. This puppy had been sick for three weeks, was in poor condition and the abdomen had a bloated appearance. The owner said the puppy had developed a cough with respiratory distress and at times would scream wildly. A kidney was removed aseptically and submitted for culture (DK 5).

MATERIAL AND METHODS

In vitro isolation from naturally infected cases

After aseptic removal of the kidneys, primary cultures were prepared by trypsinization and cells were subpassaged whenever the cells became confluent, usually between 7 and 14 days. Cultures were maintained in Hanks/Eagles medium with 10 % normal bovine serum containing penicillin and streptomycin. At times the cells were subpassaged into test tubes containing cover slips. When the cells were confluent the cover slips were stained with 10 % Giemsa or Grams stain and examined microscopically.

When the cells of the primary culture started to show signs of degeneration a suspension of a dog kidney cell line (MDCK) was added to the affected cultures. This usually occurred between the 5th and 12th passage. Cultures were examined with an inverted microscope (x48) twice weekly.

Cryopreservation

Infected tissue cultures from DK 1 were removed with trypsin citrate buffer (TCB) and suspended in Hanks/Eagles tissue culture medium with 10 % DMSO. These cells were then stored at –170 °C. After an interval of 2–5 d these cells were removed from the liquid nitrogen, thawed and placed in tissue culture flasks with medium or were added directly to an established culture of MDCK cells.

Attempted artificial infection

Encephalitozoon spores were obtained directly from infected tissue cultures. Affected cells were removed from the tissue culture with TCB and lysed with distilled water for 3 minutes in order to release spores from the cells and then restored to isotonicity. In other cases the supernatent fluid of the tissue culture was used directly. All dogs were negative to the indirect fluorescent antibody test[17] to *Encephalitozoon* prior to dosing with spores.

Experiment 1: Four puppies from 6 to 13 weeks of age of mixed breed were dosed per os with *Encephalitozoon* spores as shown in Table 1. At varying intervals the puppies were euthanazed and the kidneys removed and primary cultures prepared by trypsinization.

Experiment 2: Two pregnant serologically negative bitches were dosed per os with *Encephalitozoon* (Table 2). At varying intervals after birth four puppies were euthanazed and the kidneys removed. Primary cultures were prepared by trypsinization from the kidneys of these puppies.

Experiment 3: Three serologically negative puppies were treated with methylprednisolone* as outlined in Table 3 and varying numbers of *Encephalitozoon* spores dosed per os at the end of the immunosuppression period. At varying intervals the puppies were euthanazed and primary cultures were prepared as in the previous groups. In addition to this, part of the kidneys of two puppies were cut up into small pieces with scissors and added to a monolyer of MDCK cells. These cultures were washed 24 h later and fresh medium added.

RESULTS

In vitro isolation from naturally infected cases

Colonies of parasites could be seen developing in the cultures when examined under the inverted microscope. These intracellular parasites were seen on the second subpassage in the culture from DK 2, on the third subpassage from DK 1 and on the 1st passage from DK 3. (Puppies from Kennel A, B and C respectively.)

The number of infected cells in the cultures slowly increased until approximately 5–10 % of the cells became infected. After this they maintained this rate of infection in spite of continuous subpassage. One of these isolates (DK 2) was maintained for 82 subpassages without showing any increase or decrease in the percentage of cells infected.

Examination of the stained coverslips showed the presence of a protozoan-like parasite within intracytoplasmic vacuoles in some of the cells which were identical to Encephalitozoon[15]. Groups of spores were seen within the vacuole of infected cells. Individual spores were slightly ovoid with rounded ends. They stained with Giemsa, were Gram-positive and measured 1,08–1,42 by 2,50–2,75 μm^2.

Attempted artificial infection of dogs

Clinical signs of encephalitozoonosis were not observed in any of the 9 experimentally infected dogs (Exp 1, 3) or in the puppies of the experimentally infected bitches (Exp 2). Histological evidence of interstitial nephritis was found in all four dogs dosed directly with spores in Exp 1 (Table 1) and also in three of the four puppies from experimentally infected mothers in Exp 2 (Table 1). The remaining puppy showed only very slight lesions. The most likely cause of the interstitial nephritis in these cases would be due to successful infecion with *Encephalitozoon*.

Reisolation of the parasite from kidneys of experimentally infected dogs was successful on two occasions.

*Depo-Medrol V Upjohn

Table 1: ATTEMPTED ISOLATION OF *ENCEPHALITOZOON* FROM EXPERIMENTALLY INFECTED DOGS

Dog No.	Age (weeks)	Total spres given	Interval between dosing & culture (days)	Interstitial nephritis	Results of culture
25	12	24×10^6	107	+	Neg
28	12	24×10^6	107	+	Neg
69	6	72×10^6	50	+	Neg
23	13	5×10^6	61	+	Neg

Table 2: ATTEMPTED ISOLATION OF *ENCEPHALITOZOON* FROM PUPPIES OF EXPERIMENTALLY INFECTED DOGS

Dog No.	Age	Total spores given to dam	Pup No.	Interval from dosing to whelping	Age of pup at time culturing	Interstitial nephritis	Result of culture
60	Adult	360×10^6	A	15 d	46 d	+	Neg
			B	15 d	46 d	+	Neg
			C	15 d	91 d	+	Neg
6	Adult	5×10^6	E	39 d	8 d	Suspicious	Neg

Table 3: ISOLATION OF *ENCEPHALITOZOON* FROM IMMUNOSUPPRESSED DOGS

Dog No.	Age (weeks)	Dose Methyl-Prednisolone*	Frequency	Number of spores dosed	Interval between dosing & culture	Intersitial Nephritis	Results of culture
61	6	20 mg/kg	Daily for 3 days	72×10^6	45 d	+	+
26	12	20 mg/kg	five doses at 4 day intervals	24×10^6	108 d	+	Neg
27	12	20 mg/kg	five doses at 4 day intervals	24×10^6	108 d	+	+

*Depo-Medrol V Upjohn (Pty) Ltd

This was in two of the three dogs (number 61 and 27) (Table 3) which had been immunosuppressed with methylprednisolone in Exp 3. Parasites were first seen in cultures on the 4th and 5th subpassage respectively. Cultures of the other 5 dogs as well as of the 4 puppies from artificially infected bitches remained negative. Cultures were maintained for at least 8 subpassages before being regarded as negative. The MDCK cells to which cut up kidney had been added also remained negative.

Cryopreservation

Cryopreservation of tissue culture cells from DK 1 in liquid nitrogen resulted in a few cells attaching to the tissue culture vessel but no further growth of these cells occurred.

Addition of cells removed from liquid nitrogen to an existing cell line of MDCK cells resulted in these cells becoming infected with *Encephalitozoon*. By the second subpassage infected cells could be seen and by the sixth subpassage approximately 10 % of cells were infected.

DISCUSSION

Outbreaks of encephalitozoonosis have been described in rabbits,[4] foxes *(Alopex largopus)*[10] and captive wild carnivora at the Prague Zoo[20]. In laboratory animals the disease is often latent[12]. Previous reports of the disease in dogs has been restricted to individual dogs or in litters of puppies[1 2 7 12 14]. The present report describes severe losses in puppies from three separate kennels. No connecting link could be established between any of these kennels. The similarity of the clinical disease to distemper and other central nervous system diseases makes differential diagnosis very difficult[2]. It is therefore probably that similar outbreaks of encephalitozoonosis have been missed in the past.

It is not as yet known whether the species of *Encephalitozoon* affecting laboratory animals is the same as that affecting dogs[15]. For this reason the isolates obtained in the present study have been referred to as *Encephalitozoon* and not *E. cuniculi*. Electron microscopical studies have shown that this isolate is an *Encephalitozoon* and not a *Nosema*. A single nucleus was present at all stages of the reproductive life cycle[2].

The isolation of *Encephalitozoon* in primary tissue cultures of kidneys from an infected puppy with encephalitozoonosis has been reported[14]. The orgnism was isolted from homogenized brain and kidney tissues from the same puppy inoculated onto primary canine kidney and canine embryo fibroblast cultures. Within 10 days 50 to 75 % of the tissue culture cells were infected. In the present study *Encephalitozoon* was isolated in primary cultures in all three attempts carried out to isolate the organism from clinically infected dogs.

This successs is in contrast to attempts to reisolate the organism from dogs dosed experimentally with in-

fected tissue culture material. The presence of interstitial nephritis in histological sections of 11 dogs except one puppy (E) from which cultures were prepared would suggest that successful transmission of *Encephalitozoon* had occurred.

A number of factors could have been responsible for the failure to reisolate *Encephalitozoon* from most of the experimentally infected dogs. Natural cases with clinical signs would be expected to have more viable organisms in the tissues of the kidney than subclinical cases. The fact that organisms were reisolated from two of three dogs which were immunosuppressed could be significant in this regard. It was not however possible to estimate the number of viable organisms in the tissues in the present study.

The number of tissue culture cells which became infected with colonies of *Encephalitozoon* isolated from various hosts vary considerably between different investigators[3 8 11 14]. The type of cells used for isolation is an important factor in this regard, with rabbit choroid plexus cells producing almost 100 % infection with a rabbit isolate of *E. cuniculi*[13]. In contrast a mouse isolate grown in a mouse embryo cell line produced an infection rate of 2 %, and remained static[3]. In the present study an infection rate of from 5 to 10 % was reached after 5 to 8 subpassages. This is illustrated in the case of isolate DK 2 which reached a level of approximately 10 % of the fifth subpassage (25 d) and remained at this level. These results are contrary to those of Shadduck, Bendele & Robinson[14] who obtained infection rates of from 50 to 75 % in their cultures of *Encephalitozoon* from dogs. Their cultures included primary kidney cultures which may be comparable with those used in the present study.

The failure of infected tissue cultures to survive the freezing process is surprising. Uninfected MDCK cells frozen in liquid nitrogen using a similar technique formed monolayers without difficulty after removal from the liquid nitrogen. Cultures were frozen when between 5 % and 10 % of the cells were infected with *Encephalitozoon.* The fact that the remaining 90 to 95 % of uninfected cells were also affected by the freezing process suggests that some factor (perhaps a toxin) was released when the spores were frozen. The fact that *Encephalitozoon* could be obtained from the frozen material by adding them to MDCK monolayers indicates that at least some stages of the parasite could withstand the freezing process. These results are in keeping with other workers[8 16] who have successfully maintained *Encephalitozoon* organisms at –170 'C in Hanks medium 199 with either 10.1 glycerol[8 16] or DMSO[16] being used as a cryoprotectant. In both cases organisms were obtained by disruption of infected choroid plexuses tissue cultures.

ACKNOWLEDGEMENTS

Grateful thanks are extended to Drs J Boomker and J A W Coetzer who made the initial histopathological diagnosis in Kennels A and C.

This work was supported by a grant from the Council for Scientific and Industrial Research and from the University of Pretoria.

REFERENCES

1. Basson P A, McCully R M, Warnes W E J 1966 Nosematosis: report of a canine case in the Republic of South Africa. Journal of the South African Veterinary Medical Association 37: 3–9
2. Botha W S, Van Dellen A F, Stewart C G 1979 Canine encephalitozoonosis in South Africa. Journal of the South African Veterinary Association 50: 135–144
3. Bismanis J E 1970 Detection of latent murine nosematosis and growth of *Nosema cuniculi* in cell cultures. Canadian Journal of Microbiology 16: 237–242
4. Cox J C, Walden N B 1972 Presumptive diagnosis of *Nosema cuniculi* in rabbits by immunofluorescence. Research in Veterinary Science 13: 595–597
5. Malherbe H, Munday V 1958 *Encephalitozoon cuniculi* infection of laboratory rabbits amd mice in South Africa. Journal of the South African Veterinary Medical Association 29: 241–246
6. Marcus P B, Van der Walt J J, Burger P J 1973 Human tumor microsporidiosis. Archives of Pathology *95: 341–343*
7. McCully R M, Van Dellen A F, Basson P A, Lawrence J 1978 Observations on the pathology of canine microsporidiosis. Onderstepoort Journal of Veterinary Research 45: 75–92
8. Montrey R D, Shadduck J A, Pakes S P 1973 *In vitro* study of host range of three isolates of *Encephalitozoon (Nosema).* Journal of Infectious Diseases 127: 450–454
9. Morris J A, McCown J M, Blount R E 1956 Ascites and leptosplenomegaly in mice associated with protozoon-like cytoplasmic structures. Journal of Infectious Diseases 98: 306–311
10. Nordstoga K 1972 Nosematosis in blue foxes. Nordisk Veterinaer Medicin 24: 21–24
11. Plowright W, Yeoman G 1952 Probable *Encephalitozoon* infection of the dog. Veterinary Record 26: 381–383
12. Plowright W 1952 An encephalitis-nephritis syndrome in the dog probably due to congenital *Encephalitozoon* infection. Journal of Comparative Pathology 62: 83–92
13. Shadduck J A, 1969 *Nosema cuniculi: In vitro* isolation. Science 166: 516–517
14. Shadduck J A, Bendele R, Robinson G T 1978 Isolation of the causative oganism of canine encephalitozoonosis. Veterinary Pathology 15: 449–460
15. Shadduck J A, Pakes S P 1971 Encephalitozoonosis (nosematosis) and toxoplasmosis. American Journal of Pathology 64: 657–674
16 Shadduck J A, Polley M B 1978 Some factors influencing the *in vitro* infectivity and replication of *Encephalitozoon cuniculi.* Journal of Protozoology 25: 491–496
17. Stewart C G, Botha W S, Van Dellen A F 1979 *Encephalitozoon* antibodies in serum samples from dogs and an evaluation of the indirect fluorescent antibody test. Journal of the South African Veterinary Association 50: see this July
18. Waller T 1975 Growth of *Nosema cuniculi* in established cell lines. Laboratory Animals 9: 61–68
19. Van Rensburg I B J, Du Plessis J L 1971 Nosematosis in a cat: a case report. Journal of the South African Veterinary Medical Association 42: 327–331
20. Vavra J 1971 Nosematosis in carnivores. Journal of Parasitology 57: 923–924

THE PREVALENCE OF *ENCEPHALITOZOON* ANTIBODIES IN DOGS AND AN EVALUATION OF THE INDIRECT FLUORESCENT ANTIBODY TEST

C.G. STEWART*, W.S. BOTHA* and A.F. VAN DELLEN†

ABSTRACT: Steward C.G.; Botha W.S.; Van Dellen A.F. **The prevalence of *Encephalitozoon* antibodies in dogs and an evaluation of the indirect fluorescent antibody test.** *Journal of the South African Veterinary Association,* (1979) **50** No. 3 169–172 (En) Dept. Infect. Dis., Fac. Vet. Science, University of Pretoria, Box 12580, Onderstepoort 0110, Rep. of South Africa.

Fifteen dogs which were serologically negative to the IFA test against *Encephalitozoon* developed antibodies in their sera following experimental infection. Six other dogs which were not tested prior to infection had titres to *Encephalitozoon* after experimental infection. In a sequential study in four dogs, antibodies first appeared between 32 and 39 days after infection. These results show that the IFA test would be suitable for epidemiological studies. Fifty serum samples collected from two kennels in which encephalitozoonosis had been confirmed showed an IFA test positive prevalence rate of 70 %. In 220 serum samples submitted for various clinical pathological examinations the prevalence rate of antibodies to *Ebcephalitozoon* was 18 %.

INTRODUCTION

Encephalitozoonosis has been confirmed in South Africa in dogs[1 2 14 15], a cat[16] and laboratory animals[9]. Recently, outbreaks of the disease have been reported in breeding kennels where fairly heavy losses of puppies between 4–8 weeks of age have occurred.[14]

The indirect fluorescent antibody (IFA) test has been used in rabbits[3 4 5 6 19], foxes *(Alopex largopus)*[10] and dogs[12] to identify antibodies against *Encephalitozoon* in the serum of these animals. Other tests which have been used in rabbits are the complement fixation test[18], the immunoperoxidase test[7], a skin test[11] and a modified india-ink immunoreaction[8] with good correlation being obtained between these tests[7 8 11 18].

The isolation of *Encephalitozoon* from dogs in a tissue culture system[14] enabled us to use the IFA test to determine the prevalence of antibodies to *Encephalitozoon* and to assess its importance as a significant parasite of dogs in South Africa.

MATERIALS AND METHODS

Serum samples

Experimental Infections: A total of 15 dogs were infected with *Encephalitozoon* spores (strain DK 1) from tissue cultures as previously described[14]. Infected tissue cultures were lysed with distilled water for 3 minutes in order to release spores from the cells and then restored to isotonicity. Spores were counted in a haemocytometer. Dogs were either dosed orally or inoculated parenterally and the number of spores used in each case is shown in Table 1. Two dogs were dosed with urine from a dog which was subsequently found positive for *Encephalitozoon* on histological examination. Serum samples were collected from each dog before and after infection (Table 1) for serological purposes and stored at –17 °C until being processed for the IFA test.

In order to determine the sequential development of antibodies after infection, four dogs were infected with 24×10^6 *Encephalitozoon* spores. Serum samples were collected from these dogs at approximately weekly intervals (Table 2). To assess the effect of immunosuppression on the production of antibodies two of these dogs were injected intramuscularly 5 times at 4 d intervals with methlprednisolone* at a dosage of 20 mg/kg.

Natural outbreaks: Serum samples were collected from both normal dogs and dogs with a history of suspected encephalitozoonosis from kennels A and C.[14] Both these kennels had experienced losses in puppies from 4–8 weeks of age due to encephalitozoonosis. A fading puppy type of syndrome was most commonly observed with a few cases showing nervous signs.

Random samples: Two hundred and five serum samples were obtained from the Clinical Pathology Laboratory of the Department of Medicine, Faculty of Veterinary Science, University of Pretoria. These samples had been submitted for routine clinical pathological examination for a variety of purposes. The majority of these samples had originated from dogs in the Pretoria area.

Fifteen serum samples were also obtained from a private practitioner in Kloof, Durban. These were serum samples which had been used for routine clinical pathological examination.

IFA Test

Antigen Preparation: *Encephalitozoon* (strain DK 2) was grown in tissue cultures as previously described[14] except that LLCMK2 cells (a monkey kidney cell line) were used instead of a dog kidney cell line (MDCK). Both cell lines appeared to support the growth of *Encephalitozoon* equally well.

Spores were obtained from the supernatant fluid, washed once in phosphate buffered saline (PBS), resuspended in PBS and the volume adjusted so as to contain 8×10^6 spores per ml. Drops of glycerin were placed on a clean glass slide and sprayed with TBA** and then washed in tap water so as to leave small clear spots. A drop of PBS containing spores was then added

*Faculty of Veterinary Science, University of Pretoria.
†Major, USAF, VC. Geographic Zoonoses Division, Armed Forces Institute of Pathology, Washington D,C, 20306.
Temporary assignement: Department of Pathology, Veterinary Research Institute, Onderstepoort.
The views expressed herein are those of the authors and are not to be construed as official or as reflecting the views of the US Air Force or the Department of Defence.

*Depo-medrol V Upjohn
**TBA dry film lubricant – TBA Industrial Products Ltd.

0038-2809/79/03/0169–0172

Table 1: IFA TEST TITRES TO *ENCEPHALITOZOON* FROM EXPERIMENTALLY INFECTED DOGS

Dog number	Age (weeks)	Number of spores dosed x 10^6	Route*	Interval between dosing and post-infection test: days	Pre-infection IFA test titre	Post-infection IFA test titre
22	8	12,5	IP	90	NT	80
23	8	24	IP	100	NT	40
24	8	50	IP	100	NT	40
25	8	80	IP	100	NT	160
39	11 years	3,2	O	52	NT	320
7	5	144	IV	55	Neg	320
8	7	336	O	60	Neg	160
9	5	125	O	55	NT	40
41	2	2	O	91	Neg	160
42	2	2	O	95	Neg	20
43	2	2	O	95	Neg	320
44	2	80	O	70	Neg	320
45	2	8	O	84	10	320
46	2	8	O	80	Neg	320
48	2	80	O	105	Neg	160
37	12	Urine from infected dog	O	52	Neg	20
38	20	Urine from infected dog	O	52	Neg	80

*IP intraperitoneally; IV intravenously; O orally; NT not tested

Table 2: SEQUENTIAL DEVELOPMENT OF ANTIBODIES TO *ENCEPHALITOZOON* IN 12 WEEK OLD PUPPIES INFECTED ORALLY WITH 24 x 10^6 SPORES

Dog number	Days post infection										
	0	11	18	25	32	39	45	58	71	90	111
25	Neg	Neg	Neg	Neg	10	20	40	40	40	40	NT
28	Neg	NT	NT	Neg	NT	10	10	40	80	40	80
26*	NT	Neg	Neg	Neg	10	20	20	40	80	160	160
27*	Neg	Neg	Neg	Neg	Neg	20	40	40	40	20	40

NT = Not tested
* Puppies given 5 doses of methylprednisolone at 20 mg/kg at 4 d intervals before infection.

to each spot and allowed to dry. These antigen spots were then fixed by immersion in cold acetone (–17 °C) and stored at –17 °C until use, usually within 10 days.

Test Procedure: Antigen spots were flooded with doubling dilutions of serum and incubated in moist chambers for 30 minutes at room temperature. Both a positive and a negative serum were included in all tests as controls. The slides were then washed in PBS and flooded with a 1 in 30 dilution of fluorescein conjugated rabbit anticanine globulin** for 30 minutes. They were subsequently washed in PBS and then flooded with a 1:10 000 dilution of Evans blue for 5 minutes. After further PBS washing the slice was wetted with a glycerin buffer, covered with a glass cover slip and examined under a fluorescent microscope. Initially all serum samples were tested at a dilution of from 1:10 to 1:320/ When experience had been gained with the test all samples were screened at 1:20 and only positive samples retested at a serum dilution from 1:20 to 1:640. The titre of each serum sample was defined as the reciprocal value of the highest serum dilution showing strong peripheral fluorescence of 50 % of spores.

**Miles Laboratories.

RESULTS

In positive reactions the spores showed bright fluorescence on the periphery of the spores and on the extruded polar filament with attached sporoplasm.

Antibodies to *Encephalitozoon* were present after infection in all experimentally infected dogs (Tables 1, 2).

Dogs tested before and after infection (Table 1) showed a rise in titre following infection with the rise varying from 20 to 320. The titres prior to infection were all negative except for dog number 45 which had a titre of 10.

Four dogs bled at approximately weekly intervals after infection first showed the presence of antibodies 32 to 39 d following infection and thereafter showed a steady rise in antibody titre to between 40 and 160 within 45 to 100 d after infection. The two dogs treated with methylprednisolone showed a similar response. (Table 2).

The IFA test on serum samples collected from dogs in kennels where encephalitozoonosis had been confirmed as a cause of mortality, revealed that between 65 and 75 % of samples had a titre of 20 or greater. Seventeen of 26 dogs were recorded positive titres from kennel A and 18 of 24 from kennel C, with many of the titres occurring in the 160 to 320 range (Table 3).

Table 3: IFA TEST TITRES FROM DOGS TAKEN AT RANDOM AND FROM OUTBREAKS OF ENCEPHALITOZOONOSIS IN KENNELS

	Total number of samples	Negative	20	40	80	160	320	% positive
Kennel A	26	9	2	2	2	5	6	65
Kennel C	24	6	5	6	–	7	–	75
Durban	15	12	2	–	–	–	1	20
Pretoria area	205	168	18	7	6	2	4	18

Eighteen percent of the random serum samples collected from the Pretoria and Durban areas had a titre of 20 or greater. The majority of these titres were 20. (Table 3).

DISCUSSION

The minimal antibody titre which represents positive infection with *Encephalitozoon* is not known. The titres which developed in experimentally infected dogs, however, suggest that titres as low as 20 may be significant. Nonspecific fluorescence was sometimes detected at a dilution of 1:10; these titres were not considered to be significant.

These results suggest that the IFA test is a fairly sensitive test for the detection of prior *Encephalitozoon* infection in dogs. The fact that different strains of the parasite was used for the IFA test and experimental infections, suggests that the IFA test will detect infection with heterologous strains. Similar studies in rabbits dosed orally with *Encephalitozoon* spores showed that although some animals developed antibodies in their serum, four out of six rabbits failed to develop antibodies[17]. Parental inoculation, however, resulted in the production of high titres[17 19].

The specificity of the IFA test for *Encephalitozoon* infection in dogs has not been evaluated and therefore cross reactions with other organisms remains a possibility. Results in rabbits have shown that infection with *Toxoplasma gondii, Eimeria perforans* or *E. steidae* does not interfere with results of the IFA tests for *Encephalitozoon cuniculi*[19].

The route of infection or the number of spores dosed does not appear to have affected the subsequent titre in the present study (Table 1). The dogs inoculated intraperitoneally developed similar titres to those dosed orally. One animal dosed with 2 million spores developed a titre of 20 whereas another animal of the same age and dosed with the same number of spores developed a titre of 320. (Table 1, dog numbers 42 and 43).

The experimental dogs used in this study were infected artificially with large numbers of spores. Whether dogs are likely to ingest such large numbers of spores under field conditions is a matter for speculation. The two dogs dosed with urine are more likely to be representative of dogs infected under kennel conditions and they developed titres in the low range. The number of spores in the urine was not determined but it was probably low.

These results indicate that the IFA test shows good correlation with infection and would therefore be a useful test for epidemiological studies. A similar study of the IFA test in rabbits[4] showed excellent correlation with infection when brain and kidneys of these rabbits were subsequently examined histopathologically for lesions and *Encephalitozoon* spores and for the presence of spores in urine. No similar study has been carried out in dogs although interstitial nephritis was observed histopathologically in all the experimentally infected dogs in this study. These results will form part of a separate report.

The sequential studies reveal that antibodies are first detectable from 32 to 39 d after infection. Immunosuppression with methylprednisolone appeared to have no effect on the development of antibodies. However immunosuppression was only induced for a short period prior to infection and this could have influenced the results. The appearance of detectable antibodies was fairly late compared to similar studies in rabbits where antibodies were first detected from 7 to 28 d after infection[17].

The results of IFA tests on dogs from previously proven *Encephalitozoon* infected kennels revealed a very high number of positive sera with 70 % of samples showing a titre of 20 or more. More than half of these positives were in the titre range of 160 to 320. This high prevalence rate could be expected after outbreaks of encephalitozoonosis and the high titres obtained would suggest recent infection.

The serum samples collected from the Pretoria and Durban areas showed 18 % of samples with a titre of 20 and greater with most of them being in the lower range. Although these samples are not truely representative of a healthy dog population they should give an indication of the prevalence of *Encephalitozoon* antibodies in the general canine populaton. The reported prevalence in laboratory rabbits, rats and mice ranges from 15 to 75 % with many cases being chronic or latent[13]. In these cases diagnosis is usually by means of histopathological studies. These results however are similar to results obtained in rabbits by means of the IFA test where prevalence rates of 25 to 75 % have occurred[13].

Encephalitozoonosis has been shown to be associated with interstitial nephritis in dogs[1 2 12], its relevant importance in the aetiology of nephritis is not known.

In South Africa nephritis is a common finding in dogs and in many cases the cause is difficult to determine. In view of the relatively high prevalence of antibodies to *Encephalitozoon* shown in the present study, encephalitozoonosis should be considered in the differential diagnosis of nephritis in dogs, particularly in view of the known chronic nature of the infection.

ACKNOWLEDGEMENTS

We thank Mr H J Walzl and Dr A L Pringle who submitted the blood samples and Mrs C Sherratt for technical assistance.

This work was supported by grants from the Council for Scientific and Industrial Research and the University of Pretoria.

REFERENCES

1. Basson P A, McCully R M, Warnes W E J 1966 Nosematosis: report of a canine case in the Republic of South Africa. Journal of the South African Veterinary Medical Association 37: 3–9
2. Botha W S, Van Dellen A F, Stewart C G 1979 Canine encephalitozoonosis in South Africa. Journal of the South African Veterinary Association 50: 135–144
3. Bywaterr J E, Kellett B S 1978 *Encephalitozoon cuniculi* antibodies in a specific pathogen-free rabbit unit. Infection and Immunity 21: 360–364.
4. Cox J C, Gallichio H A 1977 An evaluation of indirect immunofluorescence in the serological diagnosis of *Nosema cuniculi* infection. Research in Veterinary Science 22: 50–52
5. Cox J C, Gallichio H A 1978 Serological and histological studies on adult rabbits with recent naturally acquired encephalitozoonosis. Research in Veterinary Science 24: 260–261
6. Cox J C, Pye D 1975 Serodiagnosis of nosematosis by immunofluorescence using cell-culture-grown organisms. Laboratory Animals 9: 297–304
7. Gannon J 1978 The immunoperoxidase test diagnosis of *Encephalitozoon cuniculi* in rabbits. Laboratory Animals 12: 125–127
8 Kellett B S, Bywater J E C 1978 A modified india-ink immunoreaction for the detection of encephalitozoonosis. Laboratory Animals 12: 59–60
9. Malherbe H, Munday V 1958 *Encephalitozoon cuniculi* infection of laboratory rabbits and mice in South Africa. Journal of the South African Veterinary Medical Association 29: 241–246
10. Mohn S F, Odegaard O A 1977 The indirect fluorescent antibody test (IFAT) for the detection of *Nosema cuniculi* antibodies in the blue fox *(Alopex lagopus)*. Acta Veterinaria Scandinavica 18: 290–292
11. Pakes S P, Shadduck J A, Olsen R G 1972 A diagnostic skin test for encephalitozoonosis (nosematosis) in rabbits. Laboratory Animal Science 22: 870–877
12. Shadduck J A, Bendele R, Robinson G T 1978 Isolation of the causative organism of canine encephalitozoonosis. Veterinary Pathology 15:449–460
13. Shadduck J A, Pakes S P 1971 Encephalitozoonosis (nosematosis) and toxoplasmosis. American Journal of Pathology 64: 657–674
14. Stewart C G, Botha W S, van Dellen A F 1979 Canine encephalitozoonosis in kennels and the isolation of *Envephalitozoon* in tissue culture. Journal of the South African Veterinary Association 50: See this issue.
15 Van Dellen A F, Botha W S, Boomker J, Warnes W E J 1978 Light and electron microscopical studies on canine encephalitozoonosis: cerebral vasculitis. Onderstepoort Journal of Veterinary Research 45: 165–186
16. Van Rensburg I B J, Du Plessis J L 1971 Nosematosis in a cat: a case report. Journal of the South African Veterinary Medical Association 42: 327–331
17. Walter T, Morein B, Fabiansson E 1978 Humoral immune response to infection with *Encephalitozoon cuniculi* in rabbits. Laboratory Animals 12: 145–148
18. Wosu N J, Olsen R, Shadduck J A, Koestner A, Pakes S P 1968 Diagnosis of experimental encephalitozoonosis in rabbits by complement fixation. The Journal of Infectious Diseases 135: 944–948
19. Wosu N J, Shadduck J A, Pakes S P, Frenkel J K, Todd K S, Conroy J D 1977 Diagnosis of encephalitozoonosis in experimentally infected rabbits by intradermal and immunofluorescence tests. Laboratory Animal Science 27: 210–216

PNEUMOCYSTOSIS: A CHRONIC RESPIRATORY DISTRESS SYNDROME IN THE DOG

W.S. BOTHA and I.B.J. VAN RENSBURG

ABSTRACT: Botha W.S.; Van Rensburg I.B.J. **Pneumocystosis: A chronic respiratory distress syndrome in the dog.** *Journal of the South African Veterinary Association* (1979) **50** No 3 173–179 (En) Dept. Path., Fac. Vet. Science, Univ. Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Pneumonia caused by *Pneumocystis carinii* in two unrelated Miniature Dachshunds is reported. The clinical findings, gross- and histopathology and some diagnostic transmission and scanning electron microscopic features of the condition are described. Although pneumocystosis has been reported from a human and a domestic goat in the Republic of South Africa, these are probably the first reported cases of the canine disease in this country.

INTRODUCTION

Pneumocystis carinii is an ubiquitous parasite of animals and man. The chronic respiratory distress syndrome caused by the filling of alveolar spaces and terminal bronchioles by this parasite can therefore be expected in many animal species. In contrast to the frequent reports of human pneumocystosis[4 8 11 20] there are but a few reports of the condition in animals. Nevertheless a wide variety of domestic and wild animal species have been affected including equines[16], swine[14], sheep[14 16], domestic goat[6], rabbit[15], guinea pigs[4], brown and white rats[1 4], wild and white mice[15] marmoset monkeys[12] cats[4], foxes[16] and dogs[2 3 17]. A peculiar and intersting finding noted by Copland[2] is that the preponderance of cases of canine pneumocystosis involved the Miniature Dachshund breed. He could not establish any connection between the pedigree of the cases reported by him in 1974 and those reported from Australia by Farrow *et al.* in 1972[3].

Sheldon was able to produce pneumocystosis in rabbits by intranasal inoculation of lung material from a fatal human case and thus demonstrated a relationship between the infection in man and animals[15]. The human disease became known as interstitial plasma cell pneumonia of premature and young infants following a detailed study of the pathology[4]. The disease is usually sporadic in occurrence but epidemics have occurred in central Europe. Gajdusek stated that more than 700 cases of human pneumocystosis had been observed in Switzerland between 1941 and 1948[4]. The disease was found to be especially prevalent in premature babies and infants 6 to 16 weeks of age, some of whom were suffering from concurrent debilitating disease4 [11]. Human pneumocystosis is, however, not restricted to infants and also occurs, invariably secondarily to predisposing conditions, in older individuals and adults. A survey conducted on human autopsies indicated greater prevalence in children less than 1 year (44 %) and those older than 60 years (11,8 %) while a low incidence (5,8 %) was found in middle aged groups[20]. Conditions such as leukaemia and primary agammaglobulinaemia, irradiation and chemotherapy used during malignant cancer treatment and organ transplants, interfere with the normal protection derived from the immune system and usually precedes *Pneumocystis* infection in adults[8 11].

P. carinii has been a microorganism of uncertain classification ever since it was found in the lungs of guinea pigs in 1909. Although originally regarded as a sporozoan and therefore a protozoan organism, the ultrastructural studies of Vavra & Kucera suggest a closer relationship to fungi to the class Ascomycetes, subclass Hemiascomycetidae[18]. Vossen *et al.* stated that the taxonomy and the developmental cycle of the organism are still uncertain[19]. In a detailed ultramicroscopic study of the developmental stages of the life cycle of the parasite they found that formation of daughter cells may occur in the so-called thin walled pneumocysts. This multiplication is responsible for the rapid increase in number of the parasites in an infected lung. They also demonstrated the presence of pneumocysts inside the alveolar epithelial cells and suggested an intracellular developmental state of the parasite. The thin-walled pneumocysts may give rise to thick-walled pneumocysts.[19]

Pneumocystosis has been reported previously from Africa, all in fact from Southern Africa[8]. Only one case has thus far been reported from an African animal – a young domestic goat[6]. This report deals with probably the first case diagnosed in dogs on the African continent.

CASE REPORTS

History and clinical findings

In Case A formalinised specimens of the lung of a 20 month old female Miniature Dachshund were received for histopathological examination. According to the practitioner concerned the dog was presented with a rapid shallow respiration which had been present for six weeks. The respiratory distress was accompanied by a bronchitis, rhinitis and moderate enteritis. The animal had not been vaccinated against distemper and it was therefore thought necessary to treat with hyperimmune antiserum* and chloramphenicol. Slight improvement was noticed and the animal was vaccinated for distemper a week later. Dyspneoa persisted persisted for another 10 days and the dog died under anaesthesia when radiographic examination of the thoracic cavity was about to be performed.

Case B was an eight month old male Miniature Dachshund from a different part of the country. Veterinary advice was sought because the animal was "panting" so much. The patient was afebrile and clinical, as well as radiological, examination did not reveal any significant abnormalities – except for the hyperpnoea. A course of penicillin and streptomycin injecions did not

Paper presented at the South African National and International Veterinary Congress, Johannesburg, September 1979.

*Anti-DHL Serum V, Fromn Lab. (Salisbury)

relieve the symptoms, neither did therapy with antihistamines or chloramphenicol. The practitioner found that oral predniselone gave some relief from the dyspnoea and hyperphoea but this beneficial effect was not obtained from using long-acting predniselone acetate administered parenterally. Cyanosis set in and the dog responded favourably to an increased dose of the oral predniselone (digitalisation did not help). Six months after the initial hyperpnoea was observed the animal was euthanased due to the persistence of the respiratory distress. A post mortem examination was carried out and specimens from the lungs were collected for bacteriological and histopathological examination.

Macroscopic pathology findings

In both cases the macroscopical pathology was reported to be limited to the lungs. In Case A both lungs appeared consolidated and had a pale yellowish-brown (caramel) discoloration. The lungs of Case B were reported to be firm in consistency with a dense consolidated appearance. No exudate could be expressed from the cut surfaces. Smears were not prepared from the lungs.

Bacterilogical results

No bacterial growth was obtained from the lung specimen submitted in Case B.

Histopathological findings

The nature and severity of the lesions in the two cases concerned were similar. The impression of a severe protein-rich alveolar oedema and patchy emphysema was gained upon low power haematoxylin – eosin stained sections. Upon closer examination all the alveoli and terminal bronchioles were found to be filled with *P. carinii* organisms which show up as a pinkish staining foamy coagulum contining small basophilic dots in some of the vacuoles (Fig 1). Exudation into the alveoli was minimal and consisted of a few macrophages, neutrophils and some desquamated epithelial cells. Focal

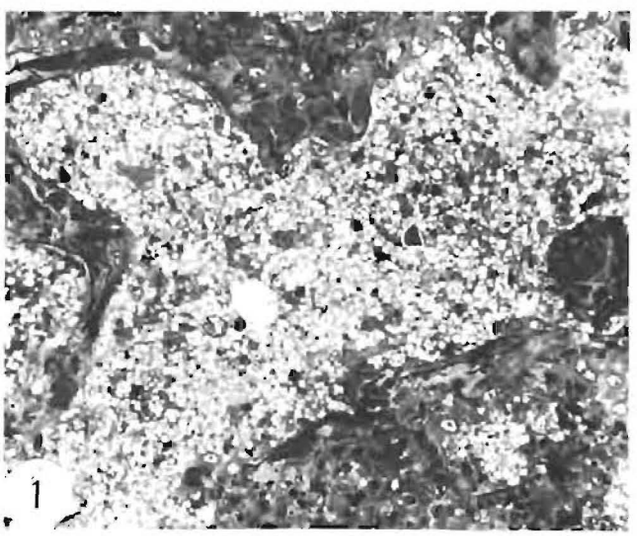


Fig. 1. Note the almost complete alveolar and bronchiolar filling by *P. carinii* orgnisms. Epon embedded, Toluidine blue stained section X175

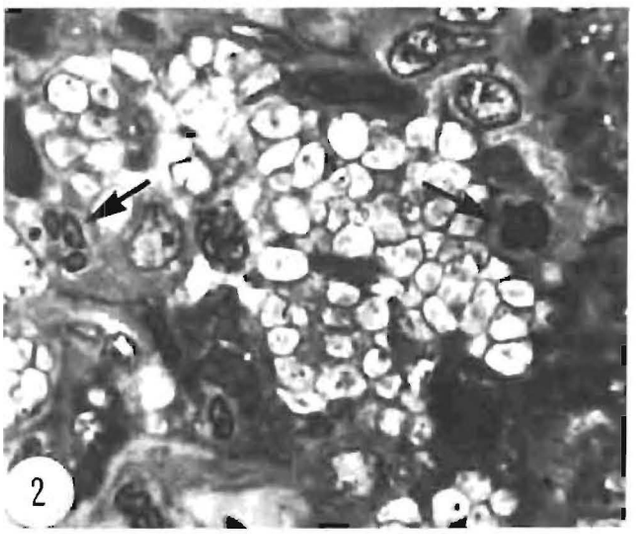


Fig. 2. Alveolar filling by pneumocysts. Two neutrophils are indicated by arrows. Epon embedded, Toluidine stained section X1750

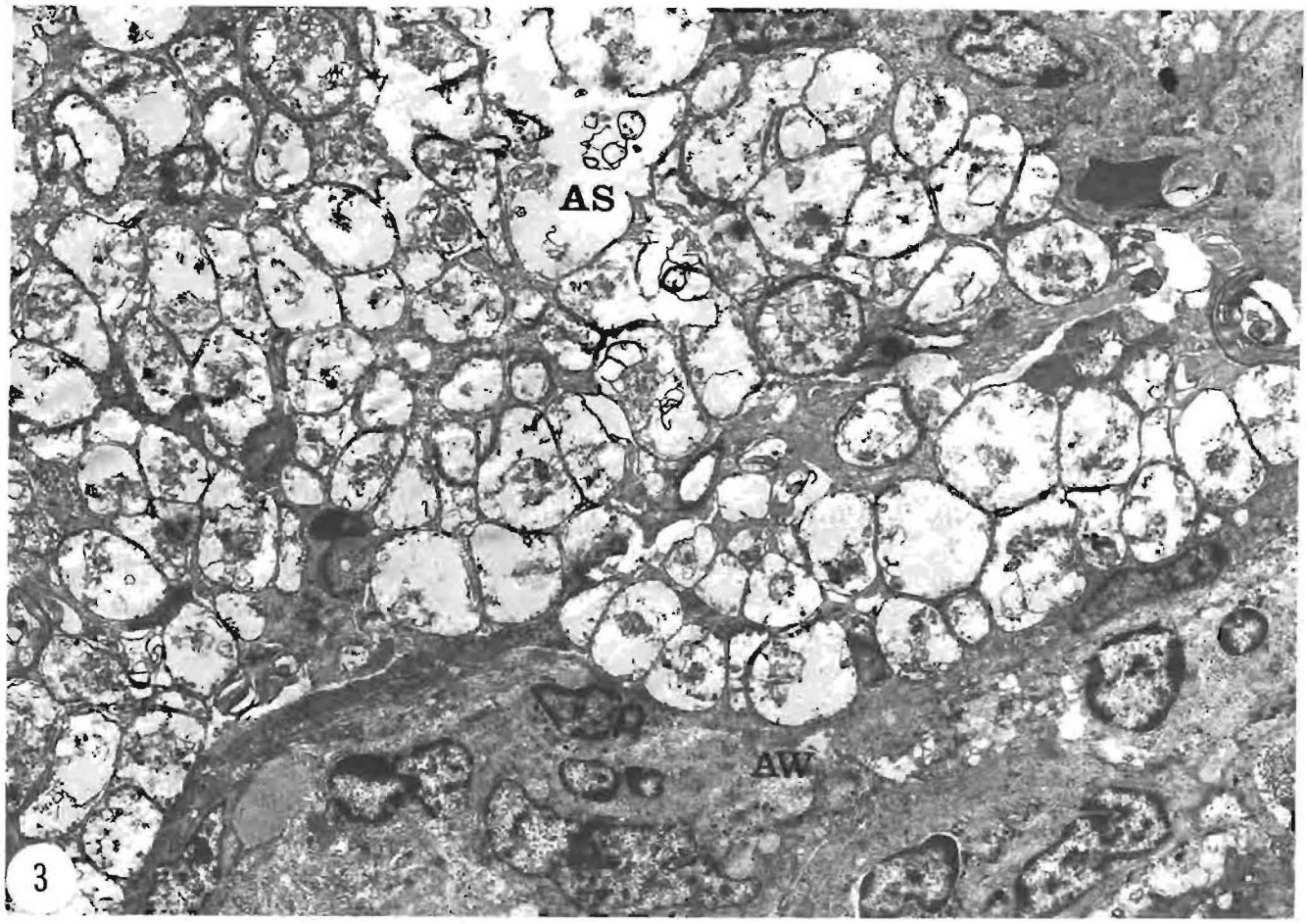
interstitial pneumonia was present and was manifested by an increased cellularity of many of the alveolar walls due to the infiltration of neutrophils, macrophages and plasma cells and the activation of the alveolar lining cells. The alveolar walls of some alveoli, although completely filled with the coagulum or organisms, were of normal appearance.

In Case B a focal area of the lung had a more marked macrophage and neutrophil reaction. These inflammatory cells were present in the lumens of alveoli and bronchioles. Some bronchi were partially filled with neutrophils and macrophages, and several of the latter contained many phagocytised *P. carinii* organisms. Vacuolated macrophages containing organisms were also present in some alveoli. Staining of lung sections by the periodic acid-Schiff's (PAAS) method gave the alveolar coagulum a bright pink honeycomb appearance due to the positive staining of the walls of the organism. In most instances the organisms appeared empty but some faint blueish bodies were present within the "cells of the honeycomb" in some instances. The walls of the organism were mildly argyrophilic on silver impregnation (Gomori's methanamine silver (GMS) staining).

Ulrastructural findings

In order to give further confirmation to the diagnosis of *P. carinii* pneumonitis some of the formalinised lung from both cases were examined under the transmission electron microscope. The delayed and non-optimal fixation used, however, rendered much of the internal structure of the organisms and cellular organelles barely recognisable.

The ultrastructural study proved an almost complete replacement of alveolar and bronchiolar space by the pneumocysts (Fig. 3). Inflammatory cells like lymphocytes, plasma cells and macrophages were recognisable in the alveolar walls. Few neutrophils could be demonstrated. The largest percentage of the pneumocysts crowded into the alvolar lumens appeared either empty or contained variable amounts of the remnants of cytoplasmic organelles such as endoplasmic reticular membranes, ribosomes and glycogen particles.



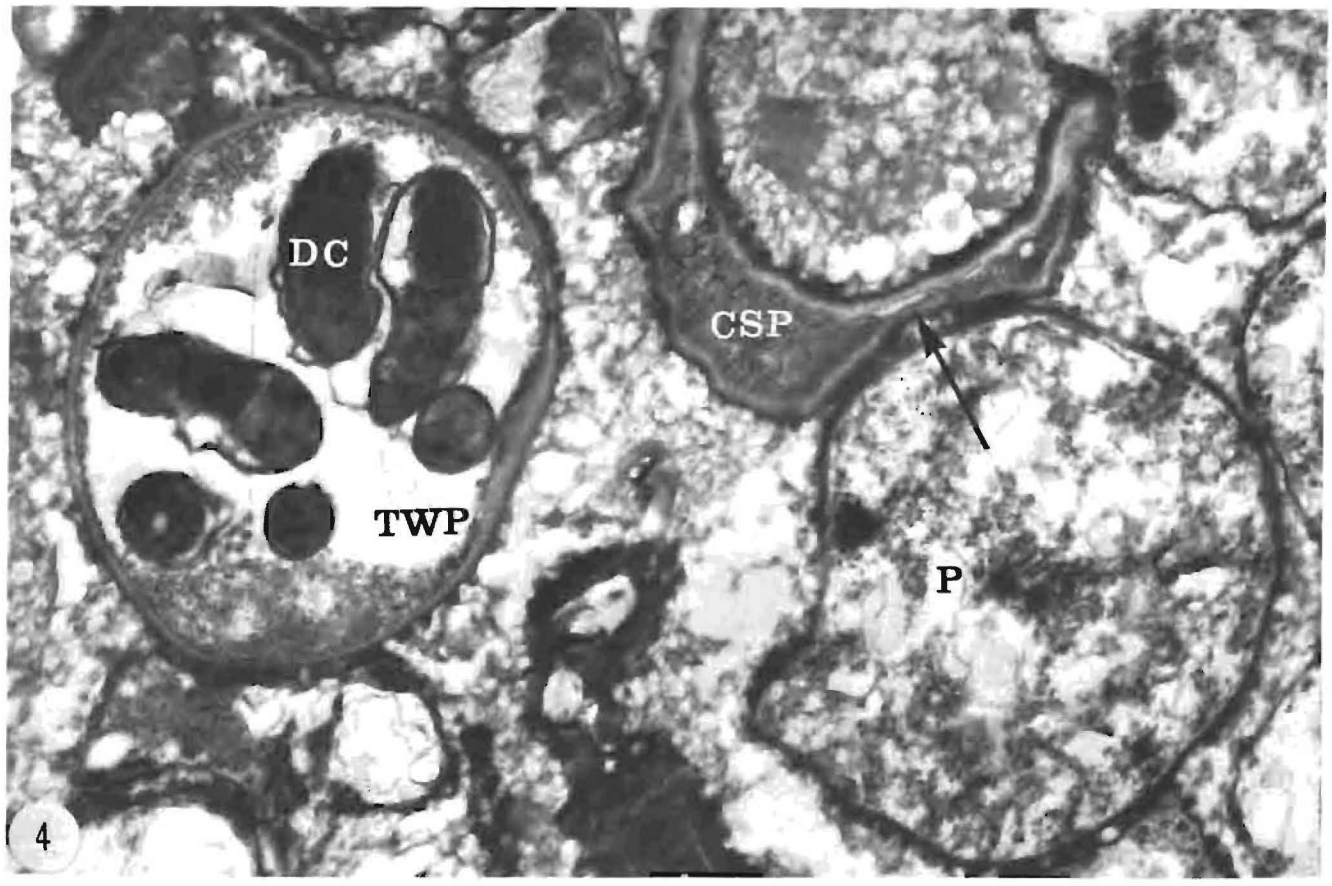
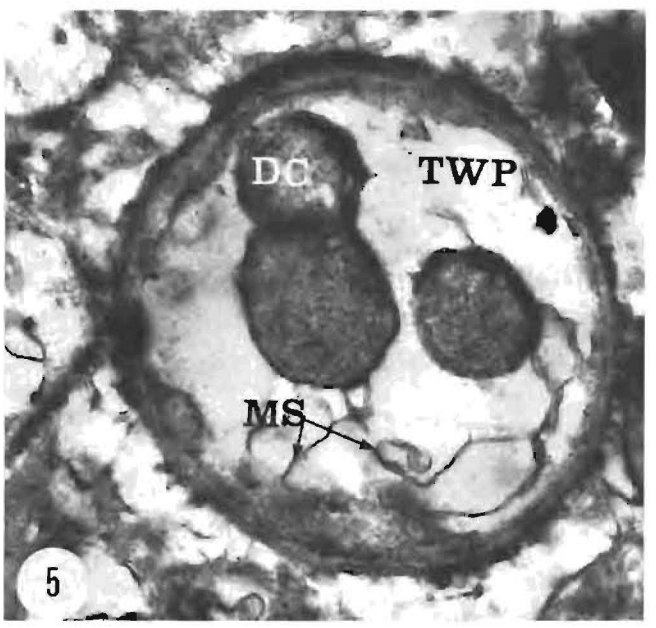
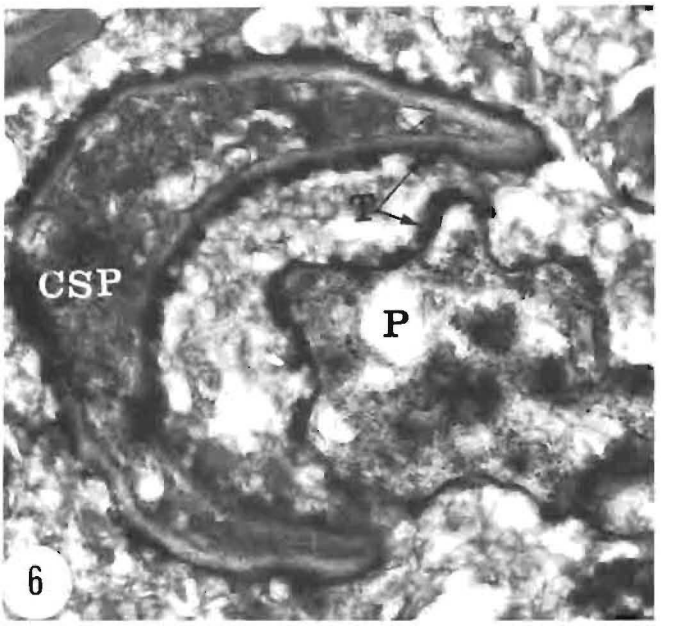


Fig. 3. Note that the alveolar space (AS) is obliterated by numerous pneumocysts many of which contain remnant material of cytoplasmic organelles; macrophages are present in the alveolar wall (AW) X3850

Fig. 4 A thick-walled pneumocyst (TWP) contains six highly electron dense daughter cells (DC) some of which are kidney shaped. A crescent-shaped pneumocyst (CSP) is indented by a thin-walled pneumocyst (P) (indicated by the arrow). X18750





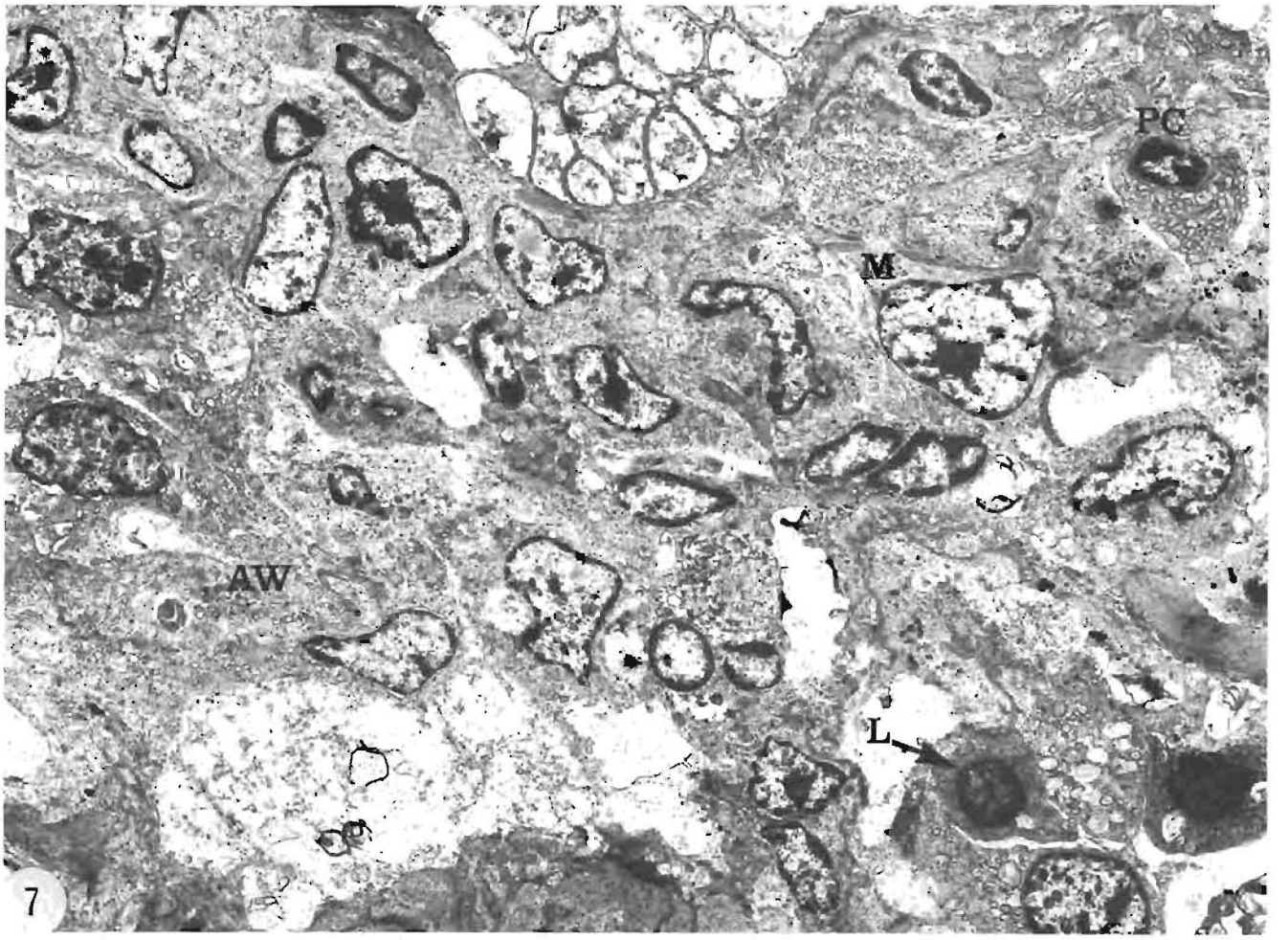


Fig. 5. A thick-walled pneumocyst (TWP) contains some daughter cells (DC) of which the walls are not fully developed Note the membrane strands (MS) that apparently connect the daughter cells and cell wall. X17350.

Fig. 6. An apparently newly liberated thin-walled pneumocyst (P) and a collapsed crescent-shaped pneumocyst (CSP) Note the tubular expansions (T) on the external part of the walls of both pneumocysts X21700

Fig. 7 The alveolar wall (AW) is thickened and contains many inflammatory cells Plasma cells (PC), macrophages (M) and lymphocytes (L) are present. X3100

Thin-walled pneumocysts varied from pleomorphic to round in shape and contained endoplasmic reticular membranes and numerous glycogen particles. The wall of these parasites measured 35–40 nm and consisted of an inner plasma membrane (the cytoplasma boundary) and an outer homogenous, moderately electron-dense layer. (Fig. 4 and 6). The thin-walled pneumocysts caused indentation of the walls of collapsed crescent shaped thick-walled pneumocysts.

Thick-walled pneumocysts containing some daughter cells in their cytoplasm were infrequently found. The parasitic cyst wall of the thick-walled pneumocysts shows great variation in thickness. The minimum thickness was 53 nm and the maximum thickness measured 159 nm. The cyst wall is composed of three layers viz. (1) a plasma unit membrane; (2) a moderate electron-transparent middle layer; and (3) an outer electron-dense layer (Fig. 4 and 5). The daughter cells were round or kidney shaped, electron dense, up to six in number and appeared to be attached to each other and the inner part of the wall of the pneumocyst by membrane strands.

The crescent-shaped pneumocysts (Fig 4 and 6) proved to be of great diagnostic avlue. These pneumocysts were thick-walled and appeared collapsed. A thin walled pneumocyst was found within the "arms" of a crescent-shaped thick-walled pneumocyst, as if it had recently excysted from the latter. All types of pneumocysts contained tubular expansions extending from their walls (Fig.6). These tubular expansions were in close vicinity of the membrane of the parasite and many were cut in cross section. They appeared as electron dense tubes which were irregularly distributed on the wall of the pneumocysts.

Scanning electron microscopy clearly demonstrated filling of the bronchial, bronchiolar and alveolar lumens with exudate containing numerous pneumocysts (Fig. 8, 9 and 10). The pneumocysts were spherical and variable in size. It was not possible to differentiate between thin-walled and thick-walled pneumocysts. The external surfaces of pneumocysts appeared irregular and the presence of interparasitic bridges were occasionally found. When compared to the loss of structural detail in the lung tissue the pneumocysts were remarkably well preserved (Fig. 9).

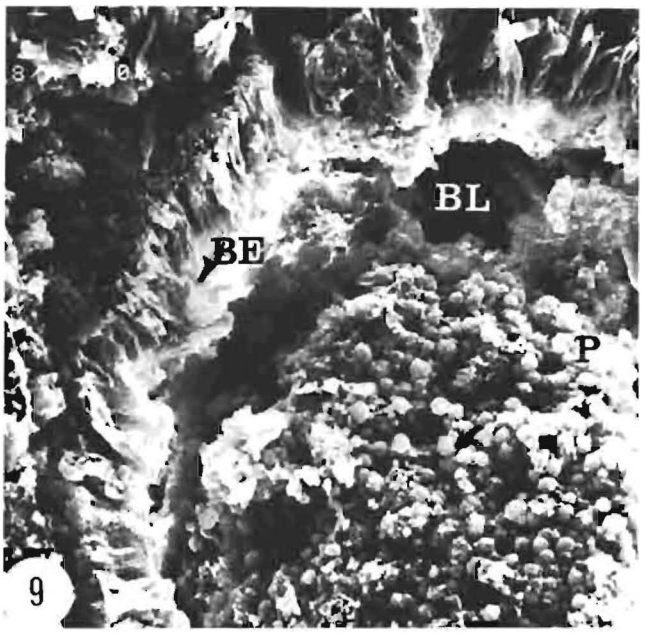


Fig. 9. Numerous pneumocysts (P) causing partial obstruction of the bronchial lumen (BL). Structural detail of the bronchial epithelium (BE) is lost. X540.

DISCUSSION

Both cases described in this report were typical of canine pneumocystosis as far as the clinical, pathological and ultrastructural findings are concerned. It is of interest to note that here, as elsewhere, the disease also occurred in pedigreed Miniature Dachshunds. Farrow *et al.* reported that all six of their cases occurred in male

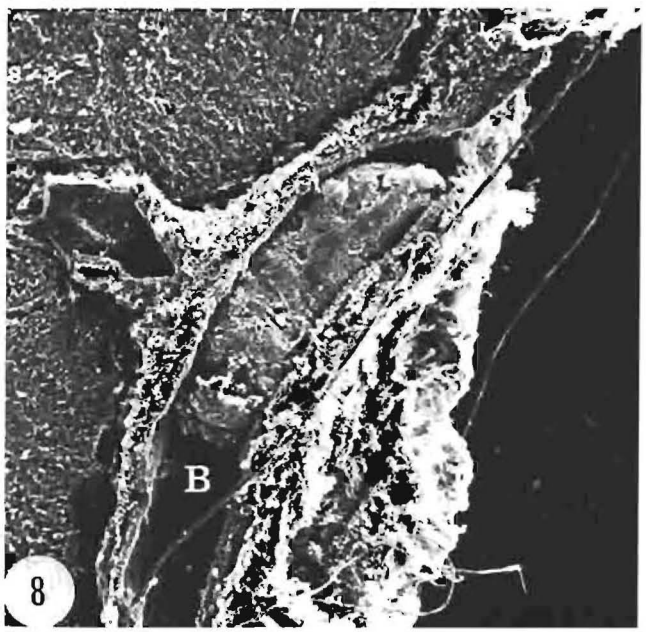


Fig. 8. Scanning electron micrograph of a small bronchus (B) in the lung of dog B. Note the plug that obstructs the lumen of the bronchus X40

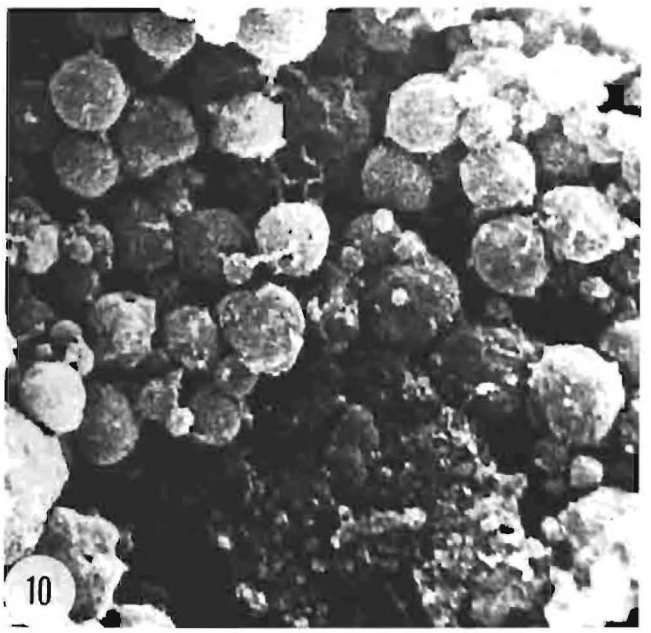


Fig. 10. Cluster of *Pneumocystis* organisms in a bronchiole. Note the marked variation in size of the pneumocysts and their spherical form. X2763.

Miniature Dachshunds, five of which were closely related[3]. They suggested that an inherited predisposition is likely[3]. This breed was also incriminated from Papua New Guinea[2]. We were not able to demonstrate any relatonship in the two cases concerned and the fact that they were both Miniature Dachshunds causes suspicion that a specific immunoincompetence may exist in this breed. This is supported by the fact demonstrated by Sheldon that immunodeficiency is important in the pathogenesis of clinical disease in laboratory rabbits[15]. Artificial manipulation and suppression of the immune system by injection of rats with cortisone acetate twice a week for longer than 46 days, caused a significant rise in murine pneumocystosis[1]. Immunoinsufficiency is also regarded as a predisposing factor in the human disease[8]11.

Pneumocystosis appears to be a sporadic disease of dogs[2 3 17] but may apparently reach endemic proportions in human infants[4]. A recent careful histopahological survey has proved the disease to be of an occult endemic nature in captive marmoset monkeys[12]. The possibility that *P. carinii* infection may also occur as a subclinical infection of dogs should therefore be kept in mind.

As far as the clinical diagnosis is concerned the hyperpnoea, dyspnoea, normal thoracic auscultation in an afebrile patient is typical. Smears prepared from tracheal and bronchial mucus or lung puncture aspirates can be useful as diagnostic aids[4]. A lung biopsy is the most reliable method to confirm the diagnosis but involves the danger of pneumothorax (percutaneous method) and the risk of general anaesthesia (open lung biopsy method) in a patient having a space occupying parasitic population in the airways of the lungs[13]. The PAS and GMS staining methods are the best for smears and sections. Upon examination of smears *P. carinii* must be differentiated from small fungi, e.g., *Histoplasma capsulatum, Cryptococcus neoformans* and *Candida albicans* which also stain positively with PAS and GMs[21]. *Torulopsis glabrata* is a yeast of the family Cryptococcaceae which is an opportunistic pathogen in man that mimics human pneumocystosis. The size, staining characteristics and the fact that these fungal organisms usually show budding and that some may have fragments of mycelial elements attached to the budding cells, help to distinguish them from *P. carinii*[21]. Zygomycete spores may also be confused with *P. carinii.* The direct fluorescent antibody test used for *Pneumocystis* is useful for differentiating *P. carinii* organisms from fungal spores[10]. A diagnostic complement fixation test for the serodiagnosis of human pneumocystosis has been developed using alcohol soluble antigen from the lungs of fatal cases of pneumocystis pneumonia[4].

Treatment with pentamidine isethionate which has long been known to be effective for human pneumocystosis, was compared to trimethoprim – sulfamethaxazole in 50 patients[5]. The therapeutic dosage of the latter was 20 mg of trimethoprim and 100 mg of sulfamethoxazole daily for a course of 14 days. The trimethoprim-sulfamethoxazole combination proved to be as effective as pentamidine isethionate and it further offers advantages such as minimal adverse effects, oral administration and ready availability[5]. Oral prednisolone gave some symptomatical relief from the dyspnoea and hyperpnoea experienced in one of our cases.

The histopathology and electron microscopy proved to be of great assistance in confirming the diagnosis since culturing of the organism is not a routine procedure. The large number of organisms causing a diffuse pneumonitis and filling of the alveolar and bronchiolar spaces, make biopsy techniques and ultrastructural examination most applicable. Extrapulmonary involvement in canine pneumocystosis appears to be rare. A fatal case of generalised pneumocystosis involving the lungs, hilar lumph nodes and heart muscle of a 9 week old sheep dog has been reported according to Gajdusek[4]. Direct pathogenicity due to the presence of pneumocystis organisms in the cytoplasm of alveolar epithelial cells has been proved[19]. The histopathogenesis of pneumocystosis has been divided in three stages, *viz* (1) an initial asymptomatic multiplication stage within the cytoplasm of alveolar epithelial cells; (2) clinical symptomatic phase with maximal parasitic replication; and (3) a final phase where the organism is destroyed by the host[11]. It is believed that thin-walled pneumocysts are found during rapid multiplication and that the formation of the thick-walled parasites might be induced by the formation of an exudate that contains immunoglobulins and complement[19]. The tubular expansions which project from the parasitic cell wall are considered to be tightly coiled membranes whose function is to hold the masses of pneumocysts together in the alveoli[18]. This study proved that some ultrastructural morphological features of the canine organism corresponds to those that have been reported in other species[1 6 11 16 18 19].

The recent success of Pifer and co-workers[7] in *in vitro* cultivation of *P. carinii* on African green monkey kidney cell cultures and the scanning electron microscopy of Murphy *et al.*[9] on organisms cultivated on monolayers of primary embryonic chicken epithelial lung cells may help to clarify the unanswered questions concerning species specificity, the probability of it being a zoonotic disease, the taxonomy of the parasite and the nature of the infection in man and animals.

Canine pneumocystosis has been confirmed in two cases in South Africa. it must therefore be included in the differential diagnosis of chronic respiratory disease of dogs especially in young Miniature Dachshunds.

ACKNOWLEDGEMENTS

Drs C C Dolman and M Terblanche are thanked for submitting Cases A and B respectively. Mr R Watermeyer and Miss H de Lange were responsible for the preparation of the electron microscopic specimens, which is appreciated. We thank Mr J Soley for assistance with electron microscopy and photography, Mr H Els for his assistance in scanning electron microscopy and the Director of the Veterinary Research Institute, Onderstepoort for the use of their microscope.

REFERENCES

1. Barton E G, Campbell W G 1969 *Pneumocystis carinii* in lungs of rats treated with cortisone acetate. American Journal of Pathology 54: 209–236
2. Copland J W 1974 Canine pneumonia caused by *Pneumocystis carinii.* Australian Veterinary Journal 50: 515–518.
3. Farrow B R H, Watson A D J, Hartley W J, Huxtable C R R 1972 Pneumocystis pneumonia in the dog. Journal of Comparative Pathology 82: 447–453
4. Gajdusek D C 1957 *Pneumocystis carinii* – Etiological agent of interstitial plasma cell pneumonia of premature and young infants. Pediatrics 19: 513–565.
5. Hughes W T, Feldman S, Chaudhary S C, Ossi M J, Cox F, Sanyal S K 1978 Comparison of pentamidine isethionate and trime-

thoprimsulfamethoxazole in the treatment of *Pneumocystis carinii* pneumonia. Pediatric Pharmacology and Therapeutics 92: 285–291.
6 McConnell E E, Basson P A, Pienaar J G 1971 Pneumocystosis in a domestic goat. Onderstepoort Journal of Veterinary Research 38: 117–126
7 Murphy M J, Pifer L L, Hughes W T 1977 *Pneumocystis carinii in vitro*. American Journal of Pathology 86: 387–394
8. Pepler W J 1958 Pneumocystis pneumonia. South African Medical Journal 32: 1003–1004
9. Pifer L L, Woods D, Hughes W T 1978 Propagation of *Pneumocystis carinii* in Vero cell culture. Infection and Immunity 20: 66–68.
0. Reinhardt D J, Kaplan W, Chandler F W 1977 Morphologic resemblance of zygomycete spores to *Pneumocystis carinii* cysts in tissue. American Review of Respiratory Disease 115: 170–172
1. Price R A, Hughes W T 1974 Histopathology of *Pneumocystis carinii* infestation and infection in malignant disease in childhood. Human Pathology 5: 737–752
2. Richter C B, Humason G L, Godbold J H 1978 Endemic *Pneumocystis carinii* in a marmoset colony. Journal of Comparative Pathology 88: 171–180
3. Rosen P P, Martini N, Armstrong D 1975 *Pneumocystic carinii* pneumonia. Diagnosis by lung biopsy. American Journal of Medicine 58: 794–801.
14. Seibold H R, Munnel J F 1977 *Pneumocystic carinii* in a pig. Veterinary Pathology 14: 89–91
15. Sheldon W H 1959 Experimental pulmonary *Pneumocystis carinii* infection in rabbits. Journal of Experimental Medicine 110: 1147–160
16. Shively J N, Moe K K, Dellers R W 1973 Fine structure of spontaneous *Pneumocystis carinii* pulmonary infection in foals. Cornell Veterinarian 64: 72–88
17. Van den Akker S, Goedbloed E 1960 Pneumonia caused by *Pneumocystis carinii* in a dog. Tropical and Geographical Medicine 12: 54–58
18. Vavra J, Kučera K 1970 *Pneumocystis carinii* Delanoë, its ultrastructure and ultrastructural affinities. Journal of Protozoology 17: 463–483
19. Vossen M E M H, Beckers P J A, M Meuwissen J H E T, Stadhouders A M 1978 Developmental biology of *Pneumocystis carinii*, an alternative view on the life cycle of the parasite. Zeitschrift für Parasitenkunde 55: 101–118
20. Weisse K, Wedler E 1954 Über das vorkommen der sogenannten *"Pneumocystis carinii"*. Klinische Wochenschrift 32: 270–271
21. Young R C, Bennett J E, Chu E W 1976 Organisms mimicking *Pneumocystis carinii*. Lancet 2: 1082–1083.

BOOK REVIEW — BOEKRESENSIE

CATTLE, PRIESTS AND PROGRESS IN MEDICINE

CALVIN W SCHWABE
DVM., MPH., ScD. University of Minnesota Press, Minneapolis 1978.
pp. xi X 263, numerous figs., Price not stated

This is a book, which, once taken in the hand, is difficult to put down. It has as its theme veterinary history, with the main purpose of putting this history into perspective and to draw therefrom certain guide lines for the future.

In the first four chapters the author examines the extent by which veterinary medicine has contributed to the advancement of human health. Ways are then considered of how this information can be used to promote future medical progress.

Early societies were mainly zoocentric as was that of Egypt at the dawn of history, when medical practice was, unexplained by historians, much more advanced that in contemporary civilisations. The author presents evidence that the knowledge of the earliest Egyptian healers was that of the body and internal workings of animals rather than that of man and that this knowledge was applied to the healing of man. This fact is taken to explain the early pre-eminence of Egypt in human medicine. Medical historians have been anthropocentric, tending to regard human and animal medicine as totally distinct with result that words like "healer" and "natural philosopher" in ancient writings have invariably been translated as "physician" with result that the basic role of veterinary or animal medicine has been overlooked. In fact the author contends that the first healers were for animals and man, i.e. they were true generalists. The first species specialists were the animal healers and only later did healers specializing in man come about.

Pursuing this theme, the author traces human and animal medical progress from Egypt to the Greek-speaking world, Italy, France, to the modern world. Many of the well-known figures in medical history are described in their veterinary context.

The era of scientific veterinary medicine and the importance of veterinary research to human health provide the main pre-occupation in the forth chapter.

The concluding chapter deals with a truly comparative medicine. Animal experimentation is a necessary prerequisite to human medical progress and can, as a very limited alternative, be replaced by propagated human or animal cells. Veterinarians therefore have a unique place in medical research. The matter of research education for veterinary and medical graduates to pursue medical research has to be investigated.

Towards the end of the book the author discusses education for research in schools of veterinary medicine and he suggests a curriculum.

It is manifestly impossible to do full justice to this book in a short review but let it be said that this very informative, well-written book is highly recommended.

C F B H

Cepravin Dry Cow.

The first Cephalosporin intramammary treatment.

Call the man from MILVET
Your business is his only business.

MILVET

Helping you help.

BBDO 00195

Milvet Ethicals (Pty) Ltd., 16 Willowton Road,
P.O. Box 936, Pietermaritzburg, 3200, telephone 41131

CARTILAGE HEALING AND REGENERATION

G.E. FROST

ABSTRACT: Frost G.E. **Cartilage healing and regeneration** *Journal of the South African Veterinary Association. (1979)* **50** No. 3 181–187 Box 50258, 2125 Randburg, Rep. of South Africa.

A study was undertaken to investigate the healing and regeneration of articular cartilage following trauma. Surgically created superficial and deep lesions involving the articular surface of the femoral condyles were evaluated in 28 dogs at intervals of two four, eight and sixteen weeks.

The general conclusion reached was that cartilage responded to trauma either with fibrous tissue repair (undergoing metaplasia to fibro–cartilage) when the lesion was deep, or by limited cellular replacement before the superficial layers became reorganised into zones resembling the normal, if the lesions were superficial. This process tended to tail off before the defect was filled i.e. the regenerative process appeared to cease before the lesion was anatomically restored to normal.

INTRODUCTION

Many workers have studied the regenerative and healing processes of injured articular cartilage. Research has shown, however, that injured cartilage displays very little propensity for primary repair even after protracted periods of time. Instead, healing occurs usually by metaplasia to fibro-cartilage of the fibrous elements growing into the lesion from the subchondral marrow spaces or from the adjacent periochondrium and fascia in the vicinity of the injured area.

Cartilage is described by Bloom and Fawcett [1] as a special type of connective tisue consisting of the cells (chondrocytes) and extra-cellular fibres embedded in an amorphous gel-like matrix. The inter-cellular components predominate over the cells which are isolated in small lacunae within the matrix.

Vaughan[2] distinguishes three ill defined layers of cells forming articular cartilage:

(a) A superficial layer in which the cells are flattened and small and lie with their long axes running parallel to the surface of the joint.
(b) An intermediate layer where the cells are somewhat larger and arranged roughly in columns at right angles to the surface.
(c) A hypertrophic layer adjacent to the bone of the epiphysis where the cells are even larger. The lowest cells in this layer become calcified and replaced by bone during the period of growth, while the more superficial cells divide by mitosis and so grow away from the bone.

Salter[3] in reviewing the literature, however, quotes Collins [4] as dividing articular cartilage more accurately into four ill defined layers, namely:

(a) A superficial zone containing small flattened cells.
(b) A transitional zone in which the cells are more nearly rounded.
(c) A deep zone with cells lying in perpendicular rows between bundles of collagen fibres.
(d) A calcified zone which is demarcated from the deep zone by a wavy line which stains blue with basic dyes. This calcified zone rests on a thin layer of subchondral lamellar bone to which it is securely attached to interdigitation and by continuity of its fibril system with the fibrils of the underlying bone.

In discussing the articular facets, Haines[5] also draws attention to the fact that hyaline articular cartilage is sharply demarcated from the calcified tissues by an undulating basophilic line, the "blue line" of Codman; the 'tide mark' or 'tideline' of Fawns and Landells[6] and Landells[7] or the basophilic line of Mankin[8].

Because of the significance of this line histologically in the response of articular cartilage to injury and its subsequent attempts at repair, as will be shown in the present study, it is preferred to refer to it as the 'basement line' instead.

Between this line, and the marrow lie the acidophilic calcified tissues.

The continuing growth of cartilage takes place by two different mechanisms:

(1) Mitoses are observed among the cells for a rather long period. After the constriction of the cytoplasm in such a division, a new partition of ground substance quickly develops and separates the two daughter cells. These in turn also divide and so give rise to the clusters of cells. This leads to expansion of the cartilage from within and is referred to as interstitial growth.
(2) The mesenchyme surrounding the cartilage primordium condenses into a special layer, the perichondrium which merges with the cartilage on one side and the adjacent connective tissue on the other. Throughout embryonic life, the cells on the inner chondrogenic layer of the periochondrium constantly differentiate into chondrocytes, secrete matrix around themselves and in this way growth on the surface or appositional growth occurs. The ability of the periochondrium to form cartilage persists but remains latent in the adult (Vaughan 1970)[2].

Apart from ageing[9] little appears to be known of biological factors such as hormones and vitamins that affect articular cartilage.

Hunter[10] observed that articular cartilage, once destroyed, was not repaired. A similar opinion was expressed more than one hundred years later by Sir James Paget[11], when he concluded that articular cartilage lacked regenerative powers and that a gap in a fracture extending into a joint, became filled with fibrous tissue. These observations were based mainly on clinical experience in observing the cartilage of joints following injury.

Mankin[10] states that after injury, one sees at first in the injured area only necrotic and atrophic changes. The defect is then filled by newly formed connective tissue which grows in from the perichondrium or from fascia in the vicinity of the injured area.

In articular injuries, the fibroblasts originate from the vascular elements of the subchondral bone if the le-

sion is deep enough. Those fibroblasts undergo metaplasia, produce capsules around themselves and become new cartilage cells – fibro-cartilage. Such metaplasia can take place in other connective tissue under the influence of simple mechanical forces acting from without e.g. pressure, especially when combined with friction associated with a low tissue oxygen tension.

Although cartilage has only a limited regenerative capacity if the cells have been damaged, the matrix components can rapidly be reformed if the cells remain intact[12].

Work done on the response to injury by Campbell[13] and Fuller and Ghadialy[14], has shown that cartilaginous injuries not extending into the subchondral bone, lacks an inflammatory component due to its avascularity, and there are minimal attempts on the part of the cartilage at cellular and matrix repair which are almost never effective in healing the defect.

Immediately after injury a relatively intense burst of mitotic activity is noted in the cartilage adjacent to the defect. This process, however, is short lived and by one week after trauma the values have diminished to levels equivalent to those of cartilage samples from normal joints.

Rosenberg (unpublished data) in a study in which articular cartilage in rabbit knees was subjected to multiple superficial lacerations, found the lacerative defects in the cartilage unaltered after one year. It was also found that there was no indication of conversion of the lesions to those of osteoarthritis or chondromalacia. This fact could have clinical significance in joint surgery where articular surfaces may be injured superficially during surgery.

Campbell[13] in discussing his experimental findings and summarizing a review of the literature on injuries of the articular surface of joints, also concludes that there is very little difference in basic concepts and that most of the earlier investigators found that injuries of hyaline cartilage healed mainly by fibrous tissue and fibro-cartilage. He does point out, however, that some workers showed in surgically produced injuries in experimental animals, that superficial injuries can sometimes heal with the formation of imperfect hyaline cartilage.

Repair by normal hyaline cartilage found by Calandruccio[15], has not been observed by others, and must be described as unusual according to Campbell[13].

The repair by ingrowth of granulation tissue is similar in type to that found around fractures, tendons and other mesenchymal tissue. From a clinical point of view Campbell[13] suggests that a fracture gap involving an articular surface be reduced as accurately as possible, with the hope that ingrowth of granulation tissue will be rapid and will transform into fibro-cartilage or hyaline cartilage. Calandruccio[15] on the other hand, suggests that if the ingrowth of granulation tissue could be prevented, it could be hoped that better hyaline cartilage would form from the superficial layers of the articular surface.

MATERIALS AND METHODS

Twenty-eight dogs were used in the study. In an attempt to achieve maximum uniformity, all the dogs had to be of the same breed, sex, approximate mass and conformation. All were to be anatomically mature. Alsation males with ages ranging from two to six years and of approximately the same size and mass were used.

In order to minimize stress and discomfort, only one hind leg per dog was to be operated on. The femorotibial joint was chosen as the femoral condyles are large and easily accessible surgically.

Prior to commencement of the study, all the dogs were clinically examined for general health. No laboratory tests were done on any of the animals. However, all eminated from a source where a high standard of hygiene was practised and where a strict deworming regimen was maintained.

As the animals became available over an eight month period individuals were selected at random and radiographs were taken of their pelvises and femurs to check on bone quality. All were found to have normal bone structures, radiographically, except that most showed signs of hip dysplasia.

The dogs were divided into four groups namely A, B, C and D (see Table 1) to be sacrificed at two, four, eight and sixteen weeks after surgery respectively.

Each group was further subdivided as follows:
Group A consisted of six dogs. Two were to be allowed free use of the affected limb after surgery when they were capable of doing so. Two more would have the affected leg immobilized in a full length plaster of paris cast post surgically. The third pair of animals in the group were to have the affected leg manually exercised for half an hour three times daily until the dogs could comfortably bear weight on it and then they would be exercised on a leash for half an hour three times daily.
Group B also consisted of the same three categories and number of dogs as Group A.
Group C consisted of eight dogs. In this group two of the animals (see Table 1) would be in plaster casts for a period of four weeks after which the casts were to be removed and the dogs allowed free movement of the limb. The fourth pair of dogs in this group (see d. Table 1) would have the affected legs immobilized for the duration of the trial. The others would be as in Groups A and B.

Under general anaesthesia the femoral condyles were approached through a lateral curvilinear parapatellar incision. The patella was dislocated medially and the stifle was flexed maximally to expose much of the articular facets of the condyles. In some instances the sub-patellar fatpad had to be incised and retraced for better exposure of the condyles. With the aid of the canula from a standard bovine ruminal trocar and canula a 9 mm diameter sharply defined round lesion was cut in one condyle as close to the weight bearing surface as possible (see Fig. 1). With the aid of a No. 15 Bard Parker scalpel blade, the articular cartilage within the circular lesion was carefully removed until the blade began grating on the deep surface of the lesion. The intention was not to penetrate the calcified layer of the cartilage or the subchondral bone plate. All the soft cartilaginous remnants were carefully removed from the lesion.

A similar lesion was created on the other condyle, only in this case a layer of cartilage estimated from the depth of the previous lesion to be about half the thickness of the hyaline cartilage, was carefully sliced away.

It was intended to alternate the deep and superficial lesion on the medial and lateral condyle. However, it was found to be easier to create a deep lesion in the la-

Table 1: CLASSIFICATION OF 28 DOGS INTO FOUR GROUPS. THE ANIMALS WERE DESTROYED AND THE JOINT LESIONS EXAMINED HISTOLOGICALLY TWO, FOUR, EIGHT AND SIXTEEN WEEKS AFTER SURGERY

GROUP A 2 Weeks post surgery				GROUP B 4 weeks post surgery				GROUP C 8 weeks post surgery				GROUP D 16 weeks post surgery			
	LESION				LESION				LESION				LESION		
Dog No.	Medial Condyle	Lateral Condyle	Category	Dog No.	Medial Condyle	Lateral Condyle	Category	Dog No.	Medial Condyle	Lateral Condyle	Category	Dog No.	Medial Condyle	Lateral Condyle	Category
26	Superficial	Deep	b	14	Deep	Superficial	c	4	Superficial	Deep	d	2	Superficial	Deep	a
27	Superficial	Deep	c	16	Superficial	Deep	c	9	Deep	Superficial	c	3	Superficial	Deep	b
28	Superficial	Deep	b	22	Superficial	Deep	a	10	Deep	Superficial	c	5	Deep	Superficial	d
29	Superficial	Deep	a	23	Superficial	Deep	a	13	Superficial	Deep	d	6	Superficial	Deep	b
30	Superficial	Deep	a	24	Superficial	Deep	b	17	Superficial	Deep	a	7	Superficial	Deep	d
31	Superficial	Deep	c	25	Superficial	Deep	b	18	Superficial	Deep	a	11	Deep	Superficial	c
								19	Superficial	Deep	b	12	Superficial	Deep	c
								20	Superficial	Deep	b	21	Superficial	Deep	a

CATEGORY:
a = Joint left free after surgery
b = Forced exercise after surgery
c = Cast for total period after surgery
c = Cast for first four weeks after surgery

teral condyle and so the majority were done in that manner (see Table I).

After the lesions were created the joint cavity was irrigated with physiological saline to remove all free cartilage remnants from the joints. The surgical incision was closed routinely.

Immediately after surgery, those legs to be immobilized were placed in plaster of paris casts from the foot to as high above the stifle joint as possible with the leg in the normal standing position.

The joints of the animals in which forced exercise was indicated, were flexed and extended for half an hour three times daily, starting on the first post operative day.

Some difficulty was experienced in immobilising the legs for periods as long as eight and sixteen weeks because the dogs chewed the casts from time to time and these then had to be renewed. It should therefore be accepted that the legs were restricted in movement rather than absolutely immobilised.

At the time of harvesting the joints, the dogs were destroyed with an overdose of barbiturate anaesthetic administered intravenously.

After fixing the specimens in 10 % formalin for 24 hours, they were decalcified in 8 % formic acid for approximately one month, during which time a fresh solution of formic acid was used every second day. The bone became soft and could readily be cut with a microtome.

Both frozen sections and paraffin sections were cut by the standard technique and the standard hematoxylin and eosin staining technique was employed in the preparation of the slides.

In creating the superficial lesion, it was attempted not to penetrate deeply into the deep zone but to remove mainly the superficial and transitional zones. It was obviously not possible to maintain such accuracy constantly as was seen in the results.

In creating the deep lesions, all soft cartilaginous tissue was carefully removed from the lesion until the grating effect of the scalpel on the calcified layer could just be felt. This left very little cartilage above the basement membrane.

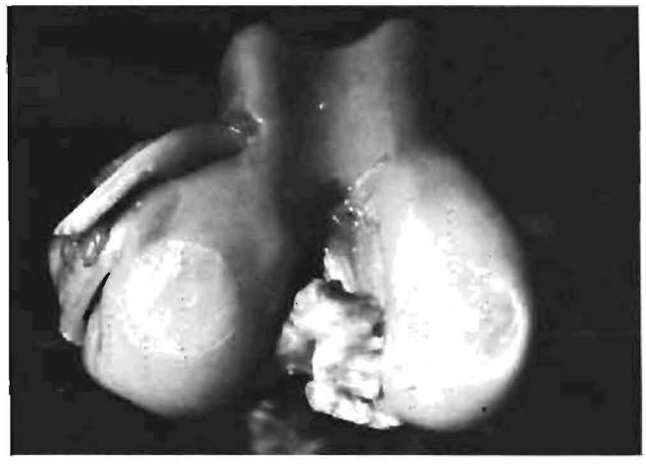


Fig. 1. Superficial lesion in lateral condyle and deep lesion in medial condyle created with the use of the canula.

RESULTS

Two weeks

After two weeks, lesions that were shallow i.e. those into the transitional zone, showed some flattening of the nuclei of the surface cells which tended to arrange parallel to the surface of the lesion. This resembled the superficial zone of normal articular cartilage: the deep columnar zone became narrower than normal as the chondrocytes forming the isogenous groups towards the outer margin of this zone became separated from one another and became more rounded and typical of the cells of the transitional zone.

The overall appearance was one of the zones taking on a more or less normal appearance but with the zones

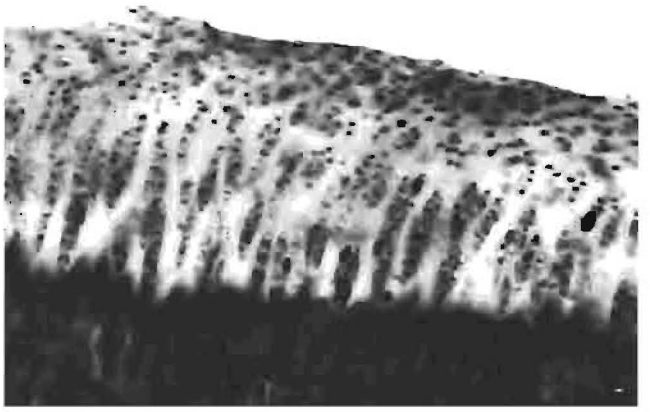


Fig. 2. Photomicrograph of superficial lesion after two weeks. Note the realigning of flattened cells parallel to the surface. The transitional and deep zones are reduced in thickness. (x 150)

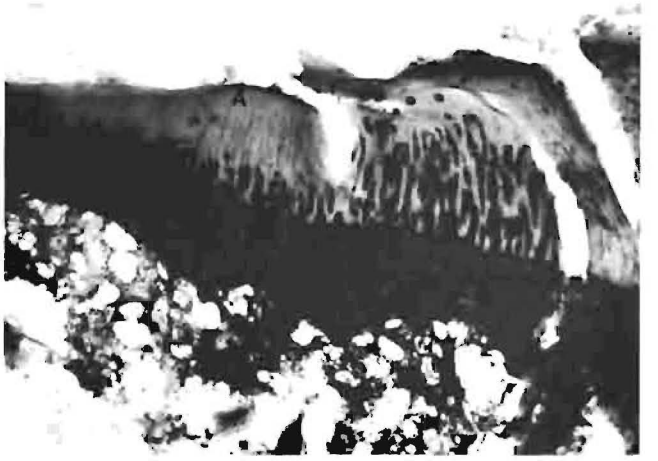


Fig. 3(a). Deep lesion. Note the infolding of the edge of the lesion. Part of the deep zone has remained viable, especially where protected by the infolding edge. Near the surface cell death has occurred, leaving "ghost" lacunae. (A) (x 60)

narrower than normal, leaving a saucer shaped depression i.e. the reparative process appeared to level off before the defect was filled (see Fig. 2).

Where the lesion was slightly deeper i.e. into the deep zone, the columns of cells appeared static and unaltered except that the staining reaction was much more basophilic (H.E.).

The edges of the lesion constantly folded inward to form a smooth transition from normal to injured areas.

Where the lesion did not penetrate beyond half the thickness of the cartilage layer, the columns of chondrocytes remained viable, especially at the edge of the lesion where some protection was provided to underlying layers by the infolding of the edge of the lesion. As the lesion became deeper, cell death in the superficial layer occurred as indicated by 'ghost' lacunae with pale staining cytoplasm and loss of nuclear material. In the depth of the lesion where the layer of matrix above the basement line was thin, all chondrocytes had disappeared and the matrix appeared to be sloughing from the basement line (see Fig. 3a and b).

Although the calcified zone below the exposed basement line did not differ from the adjacent normal, there were signs of increased vascularity and cellularity of the subchondrial marrow spaces in some sections. Tongues of trabecular bone were extending toward the basement line but did not reach it.

The typical collapse of the edges of the lesion was evident in all sections creating and even slope to a saucer shaped lesion.

Evidence of cell division was present at the edges of the lesion as indicated by clusters of nuclei in individual lacunae in these areas (see Fig. 4).

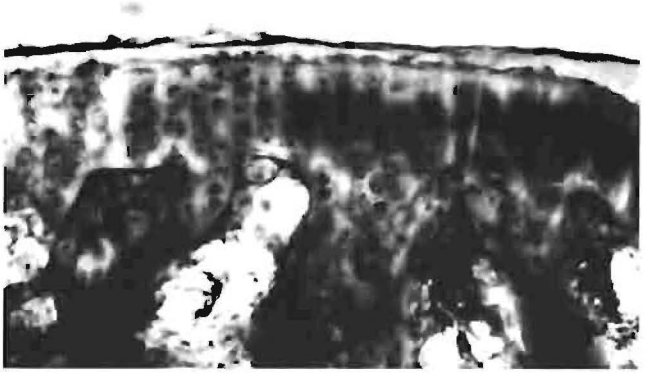


Fig. 3(b). In the depth of the lesion all cells have disappeared above the basement line and the remaining matrix is disintegrating. Increased cellularity of the marrow spaces is evident. (x 150)

Four weeks

The unfolding of the edges of the lesion was again evident. Where the lesion was not deeper than the transitional zone, the cells near the surface, although sparser than normal, tended to arrange parallel to the surface of the lesion to some extent. This realigning of the surface cells did not occur when the lesion was into the deep zone. There was a definite loss of cell numbers especially when the lesion extended into the deep zone.

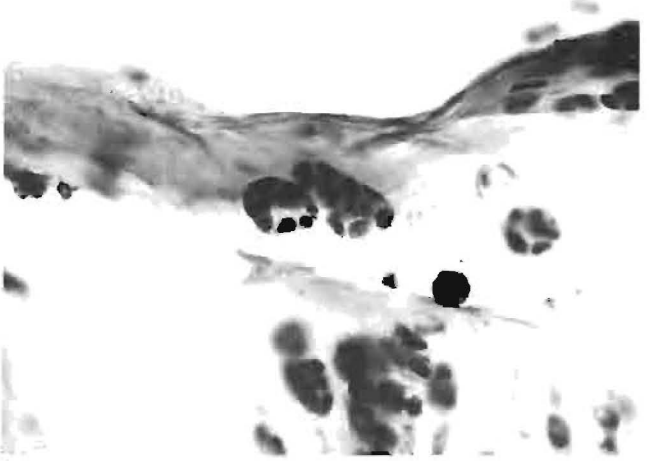


Fig. 4. Enlargement of Fig. 3(a), showing clusters of nuclei in lacunae indicative of active cell division. (x 300)

Where the basement line was disrupted and the calcified zone was damaged in creating the lesion, there was a marked fibroplastic response from the subchondral bone with connective tissue growing into the lesion. Where the basement line was not disrupted, and the lesion was shallow, no cellular reaction in the subchondral marrow spaces was evident.

In deep lesions, loss of cells and matrix in the depth of the lesion exposed the basement line.

The marrow spaces became very cellular and buds of trabecular bone approached the basement line and even reached it in spots. However, there was no breakthrough of fibrous tissue from the subchondral marrow spaces where the basement line was intact. The ingrowth of tongues of trabecular bone was thus eroding and replacing the calcified zone.

Eight Weeks

In the superficial lesions cell loss was evident by an increased ratio of matrix to chondrocytes as compared to adjacent normal cartilage. The remaining cells appeared viable and were probably multiplying as the isogenous groups of cells present were different from those in the adjacent normal cartilage. Seen especially near the edge of the lesion were lacunae with multiple nuclei which indicated cell division (see Fig. 5).

The characteristic infolding of the edges of the lesion was evident.

Where the lesion extended only to the transitional zone, the surface cells tended to allign themselves parallel to the surface.

In the deep lesions the matrix was found to slough from almost the entire bed of the lesion onto the basement line. The basement line also tended to stain more deeply basophilic (H.E.).

A marked increase in cellularity of the subchondral marrow spaces was evident with tongues of trabecular bone penetrating the calcified zone towards the basement line and reaching it in places (see Fig. 6). There were also areas of breakthrough with connective tissue ingrowth which appeared to undergo metaplasia to fibro-cartilage. The bonding of the fibro-cartilage to the pre-existing hyaline cartilage at the edge of the lesion appeared firm, with the surface cells lying parallel to the joint surface (see Fig. 7).

Sixteen weeks

Where only the superficial and transitional zones were destroyed, some flattening out of the remaining superficial cells was evident. These came to lie parallel to the surface. The deep zone was decreased in thickness with no apparent mitotic activity which would indicate regeneration of this zone.

Increased ratio of matrix to cells near the surface, was indicative of cell loss. However, some cell division could be presumed by some large lacunae filled with numerous nuclei. These would occur near the surface of a lesion especially in the thicker cartilage near the edge of the lesion.

Where the calcified zone was damaged and the basement line disrupted, fibrous tissue from the subchondral marrow spaces overgrew the remaining hyaline cartilage in the lesion to form a firm bond between itself and the hyaline cartilage.

In the deep lesions the tissue loss above the basement line was complete. Where the basement line was intact,

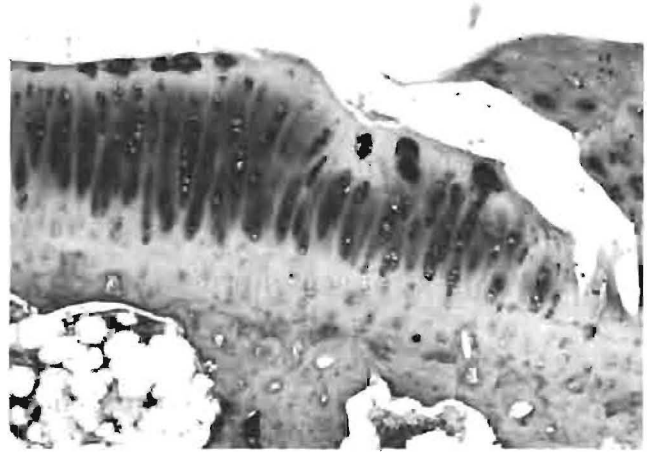


Fig. 5. Photomicrograph of superficial lesion after 8 weeks. Note the infolding edge of the lesion and cell division occurring at the surface of the lesion. (x 60)

Fig. 6. Deep lesion showing complete loss of cartilge above basement line, and subchondral bone buds reaching the basement line from below. (x 60)

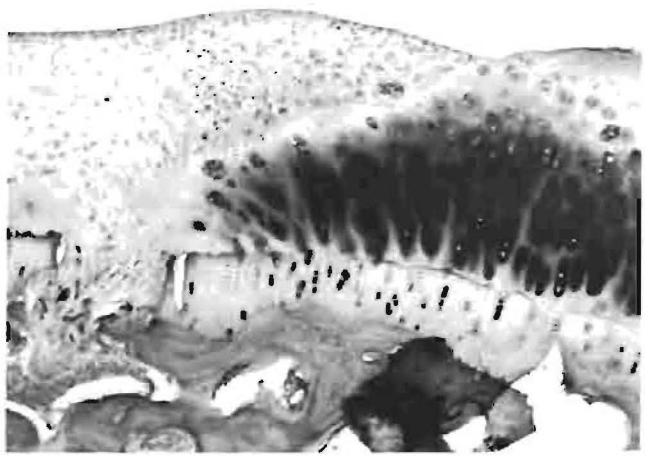


Fig. 7. Connective tissue from the subchondral marrow spaces invading the lesion and undergoing metaplasia to fibrocartilage. Note the firm bonding with hyaline cartilage at the edge of the lesion. (x 60).

the subchondral buds of trabecular bone had eroded the calcified zone to reach the basement line. Where these bone buds eventually penetrated the basement line, fibrous tissue broke through to initiate fibro-cartilage healing of the lesion.

An increase in cellularity of the marrow spaces was present in all sections.

DISCUSSION

One of the objects of the study was to establish what effect, if any, movement and immobility of the affected joint would have on regeneration and healing of damaged articular cartilage.

After studying the histological sections, it became clear that there were no apparent deviations from a constant histological response which was present in all the categories viz. free use of the leg, immobilization of the limb for part or all of the duration of the trial and forced exercise of the affected limb.

It was accepted that only two specimens per category in each group was too small a number to make a statistical analysis meaningful. However, if one took into account the fact that in every group from two to sixteen weeks post surgery, the histological response was practically identical in every dog irrespective of what category it fell into, then it was reasonable to conclude that in the present study neither forced or passive movement of the injured joint nor reduced mobility, had any effect on the reparative process.

The 'blue line', 'tide mark' or 'tide line' described by others and referred to as the 'basement line' in this study, was found to be significant histologically in the response of articular cartilage to injury.

When articular cartilage was removed to a depth of about two thirds of the columnar deep zone, the remaining tissue appeared unable to remain viable and the chondrocytes disappeared leaving only a pale staining matrix which began to disintegrate and slough from the underlying basement line. (Where the remaining layer was thicker, the chondrocytes tended to remain viable and did show signs of multiplying). This phenomenon was present as early as two weeks post injury, became more marked at four weeks and by eight weeks practically the entire bed of the lesion showed an exposed basement line. Lack of support of the chondrocytes in the unstable remaining matrix and possible diffusion deficits of nutrients in the damaged tissue were possible causes of the cell death which in turn led to the loss of matrix which was secreted by these cells.

The metaplastic calcified zone was found to be much more stable, the normal structure was maintained and it did not disintegrate from the surface. However, when the basement line became denuded of cartilage, a subchondral bone response was triggered. Tongues of trabecular bone began replacing the calcified zone and extended towards the basement line at two weeks. There were also signs of increased cellularity (fibroblasts) and vascularity of the marrow spaces. The tongues of trabecular bone did not reach the basement line, however, and there was no ingrowth of fibrous tissue into the lesion unless the basement line had been disrupted in creating the lesion. Where this had occurred, even at two weeks post surgery, an active fibrous tissue response in the lesion was already evident.

By four weeks post surgery trabecular bone buds had reached the basement line in spots, but with this line intact no fibrous tissue breakthrough occurred spontaneously.

After eight weeks the process of necrosis of chondrocytes and sloughing of matrix left the basement line exposed along almost the entire bed of the lesion. The line stained more deeply basophilic (H.E.) than before. The increase in cellularity of the subchondral marrow spaces was marked at that stage and the trabecular bone tongues reached the basement line at numerous points and the process had actually progressed to spontaneous breakthrough of the trabecular bone through the basement line allowing fibrous ingrowth into the lesion. The fibrous tissue formed a complete union with the hyaline cartilage at the edge of the lesion. Metaplasia of this tissue to fibro-cartilage would result.

By sixteen weeks the tongues of subchondral bone had reached the basement line at many points and by destroying it, allowed an ingrowth of fibrous tissue to replace the line and calcified zone.

The above sequence of events showed that once cartilage destruction reached the basement line, the vascular elements of the subchondral bone were activated to initiate the well known fibro-cartilage healing of the defect.

Previous workers have ascribed the subchondral bone response in injury of the articular cartilage, to the effect of pressure on the denuded bone plate. In the present study the lesions created in the articular surfaces were large and at least part of the lesion would not have been subjected to the stress of weight bearing. Also, there was no detectable difference in response in the different categories in the study. It is suggested that the inflammatory response of the subchondral bone could be at least partly due to other factors such as biochemical changes occurring in the calcified zone when the surface layers were removed. This aspect has to be further evaluated.

When the lesion was not deep, tissue death to the basement line did not occur and the subchondral bone was not stimulated.

The incomplete repair process in these lesions consisted of the remaining layers of hyaline cartilage attempting to reform the different zones of normal cartilage. However, this occurred before the slowly dividing chondrocytes could fill the lesion to the normal joint contour. It appeared that once the superficial layer of cells arranged themselves parallel to the surface, the process of repair of the defect stopped, thus creating the impression that the lesion had not healed. It may be that due to the lack of an inflammatory component in the healing process, and because articular cartilage lacks a definite limiting membrane found in other tissues, e.g. epithelium, capsules, periosteum etc., which are important in guiding the healing process, this process is not only much slower, but also terminates more unpredictably in articular cartilage than in other tissues.

In all superficial lesions cell loss near the surface was evident but it appeared that once the cells on the surface became flattened and parallel to the surface in a layer, it had a stabilizing effect on the underlying chondrocytes and matrix. It also appeared that if the deep columnar zone was mainly intact, the superficial layer of this zone gave rise to new but narrower transitional and superficial zones. If part of the deep zone was also destroyed very little regeneration was noticed and no

sign of increased cell division was evident in the isogenous columns of chondrocytes.

A constant finding in the present study was a characteristic infolding of the edges of the lesion to give rise to a gradual slope from the edge to the depth of the lesion, thus creating a smooth saucer shaped depression. This reaction was already present two weeks post surgery. This too has been ascribed by other workers to the effects of pressure on the edge of the lesion, forcing it to collapse inward. It is suggested, however, that the phenomenon is an active process initiated by 'flowing' of the matrix at the edge of the lesion, thus redirecting the direction of growth of the chondrocytes into the lesion rather than perpendicular to the articular surface.

The results obtained in the study indicate that articular cartilage responds to injury in one of two ways depending on the degree of damage. In injuries that are superficial, the remaining cartilage cells multiply, but the process is slow and incomplete, tailing off before regeneration is complete anatomically. However, functionally the healing process appears to be more complete in that the remaining cartilage layer tends to reestablish itself into superficial transitional and deep zones. Although these zones are narrower than normal, they do resemble the normal histologically. It has been pointed out by other workers that such superficial lesions do not give rise to pain or arthritis.

It would require long term studies (in excess of one year) to establish if these saucer shaped superficial lesions do eventually heal to the extent of restoring the normal anatomical contour of the articular surface. It is possible that once the superficial and transitional zones have reformed, the genetic stimulus controlling the healing process is removed. The resultant defact would then be permanent.

Lesions beyond a certain critical depth lead to progressive necrosis of the remaining chondrocytes of the layer above the basement line. No primary healing occurs in these lesions and the vascular elements from the subchondral marrow spaces penetrate the lesion by eroding the calcified zone and breaking through the basement line. These lesions are filled with fibrous tissue which undergo metaplasia to fibro-cartilage – an inferior substitute which can be compared to the scar in the healing of other tissues.

REFERENCES

1. Bloom W & Fawcett,D W 1975 A Textbook of Histology. 10th Ed., Philadelphia, London, Toronto. W B Saunders Company
2. Vaughan Janet M. 1970 The Physiology of Bone. Oxford. Clarendon Press
3. Salter R B & Field P 1960 The effects of continuous compression on living articular cartilage (an experimental investigation). The Journal of Bone and Joint Surg. 42A: 31–49
4. Collins D H 1940 The Pathology of Articular and Spinal Diseases 19–23, London. Edward Arnold & Co
5. Haines R W & Mohuiddin A 1968 Metaplastic bone. Journal Anatomy 103: 527–538
6. Fawns A T & Landells J W 1953 Histochemical studies of rheumatic conditions. I. Observations on fine structures of the matrix of normal bone and cartilage. Am rheum. Dis 12: 105–113
7. Landells J W 1957 The reactions of injured human articular cartilage. Journal of Bone and Joint Surgery 39B: 548–562
8. Mankin H J 1963 The calcified zone (basal layer) of articular cartilage of rabbits. Anatomical Record 145: 73–88
9. Mankin H J & Baron P A 1965 The effect of ageing on protein synthesis in articular cartilage of rabbits. Laboratory Investigation 14: 658–664
10 Hunter W 1743 On the structure and diseases of articulating cartilage. Phil Trans. B.9: 267
11. Paget J 1853 Healing of injuries in various tissues. Lectures on Surgery and Pathology I: 263
12. Mankin H J 1974 The reaction of articular cartilage to injury and osteoarthritis. The New England Journal of Medicine 12: 1285–1290 and 1335–1340
13. Campbell C J 1969 The healing of cartilage defects. Clinical Orthopaedics. 64: 45–63
14. Fuller J A & Ghadialy F N 1972 Ultrastructural observations on surgically produced partial thickness defects in articular cartilage. Clinical Orthopaedics 86:
15. Calandruccio R A, Gilmer S G 1962 Proliferation, regeneration and repair of articular cartilage of immature animals. Journal of Bone and Joint Surgery 44A: 431.

HAND-REARING OF CAPE HUNTING DOG *LYCAON PICTUS* PUPS

J. VAN HEERDEN

ABSTRACT: Van Heerden, J. **Hand-rearing of Cape hunting dog *Lycaon pictus* pups.** *Journal of the South African Veterinary Association* (1970) **50,** (En) 189–191. Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, Onderstepoort 0110. Rep of South Africa.

Problems encountered in the hand-rearing of Cape hunting dogs are discussed. It is felt that these could be minimised by avoiding stress, by rearing pups as close to natural circumstances as is possible, by applying sound husbandry techniques and by keeping the pups together.

INTRODUCTION

This paper deals with an attempt to rear Cape hunting dog *(Lycaon pictus)* pups under highly artificial conditions. Six puppies, about six weeks old, were salvaged from their den in the Gravelotte area of the Transvaal Lowveld. (A salvage operation outside a National Park) They were then transported to Pretoria (Transvaal) where they were housed in a closebox stable with ample bedding covering the floor. Bales of *Eragrostis tef* were put inside the stable and were partly shaped into a shelter. During daytime the pups had access to a small open pen planted with kukuyu-grass. *(Penissetum clandestinum).*

Procedures and Observations

During Days 1–6 the pups were not handled at all. Food and water were offered in clean metal bowls. The food was presented in a semiliquid form and consisted of a mixture of a commercial cereal*, minced meat and cows milk. This mixture was presented twice daily. The appetite of the puppies remained satisfactory, until the sixth day when the presented food was not finished. Very little if any water was taken during this period. On the Day 7 food was left untouched and the pups stayed inside their nest. They were then caught and found to be dehydrated, with faeces-stained hindlegs. A green slimy diarrhoea was observed in all the pups. A course of supportive treatment was immediately instituted. This consisted of subcutaneous and oral fluid administration**, antibiotics† and anti-diarrhoeal‡ treatment. Oral dosing was facilitated by the use of a stomach tube. Faeces were examined for internal parasite infestation.

On Day 8 a severe, some-what projectile, green watery diarrhoea was evident and three of the puppies were severely dehydrated. Supportive fluid and other treatment was given every four hours. In some pups the intravenous route had to be used.

Three pups died on Day 9 and a full post-mortem examination was done. The remaining ones were kept alive on subcutaneous and oral fluid administration for the ensuing five days. The diarrhoea stopped but the faeces were still fairly loose. During this period all food except for a few licks of the cereal was refused.

Over the following eight days the pups were offered vomitus and some cereal. Vomitus was obtained by starving mature domestic dogs overnight. In the morning they were fed meat and approximately two hours later prompt vomition was induced by placing ½ a tablet of apomorphine§ under the lower eyelid. (This diet was later supplemented with fresh meat). Although their appetites had by now almost returned to normal the pups were potbellied and thin with a dull and shaggy coat. Alopecia and a papular dermititis developed on the dorsal aspect of the head in all three pups. The faeces were still very loose. Haematological and blood chemistry tests on Day 22 showed the pups to be anaemic and hypoproteinaemic.

The pups were now bathed twice weekly for 4 weeks using a commercial shampoo*. From Day 23 onwards the pups were put onto a diet of meat and fresh cow's milk containing egg. The latter was accepted within a few days and was eventually taken very readily. Meat was offered in various forms, such as partially plucked chickens, lengths of sheep and bovine intesting, pieces of bovine and ovine liver, heart, spleen and muscle as well as mice, rats and guinea-pigs. Calcium and Vitamin D was supplemented†. Eventually only sheep and bovine carcass material was used. (See Table 1).

Table 1: EXTRACT FROM MENU FOR THREE WILD DOG PUPS (From Day Fifty to Day Fifty Seven)

Monday:	am:	1 kg meat 1 litre milk + 1 egg
	pm:	1 kg meat
Tuesday:	am:	1.4 kg meat 1 litre milk + 1 egg
	pm:	1.2 kg meat
Wednesday:	am:	1.2 kg meat 1 litre milk
	pm:	1 kg meat
Thursday:	am:	1 kg meat + 1 litre milk + 1 egg
	pm:	1.4 kg meat
Friday	am:	1.2 kg meat + 1 litre milk + 1 egg
	pm:	1.4 kg meat
Saturday:	am:	1.4 kg meat + 1 litre milk
	pm:	1.2 kg meat
Sunday:	am:	1.4 kg meat + 1 litre milk
	pm:	1.3 kg meat

All meats were either sprinkled with ICD granules or Calsuba.

*Pro-Nutro. Hind Bros & Co. Ltd., Murray road, Wadeville, Transvaal.

**Plasmalyte B; Sodium Chloride 0,9 % (m/v) and Dextrose 5 % (m/v) injection B.P.; Ringer-Lactate Solution. Baxter Laboratories, Deerfield, Illinois, USA.

†Penbritin. Beecham Animal Health, Brentford, Middlesex, England.

‡Dia-Stat 500. Propan Ethicals, P O Box 10534, Johannesburg.

§Apomorphine hydrochloride, Centaur Labs., 22 Buxton street, Doornfontein.

*Elizabeth Anne's Special Baby Shampoo, Elizabeth Anne, PO Box 41498, Craighall, S.A.

†Calsup, Centaur Labs, Buxton Street 22, Doornfontein.

‡Calsuba Group Laboratories SA, 21 Wrench Rd 21, Isando, Tvl.

ICD-Milborrow & Co., Pty Ltd., 16 Willowton Road, Pietermaritzburg.

0038-2809/79/03/0189–0191

Table 2: RESULTS OF HAEMATOLOGICAL AND BLOOD CHEMISTRY TESTS

	Day 22		Day 36			Day 200		
Pup no.	1	2	1	2	3	1	2	3
Hb (g/l)	97	110	104	100	109	162	145	152
RCC (10^{12}/l)	4,64	4,84	5,47	4,89	5,66	7,72	6,94	6,99
Ht	0,28	0,31	0,40	0,38	0,41	0,56	0,48	0,50
MCV f	–	–	–	–	–	64	63	64
WCC (1 x 10^9/l)	23,9	24,6	20,5	17,1	13,3	16,1	16,3	13,2
Neutrophiles	0,97	0,86	0,83	0,88	0,87	0,73	0,67	0,68
Lymphocytes	0,01	0,04	0,05	0,05	0,01	0,18	0,19	0,19
Monocytes	0	0,03	0,06	0,03	0,10	0,02	0,05	0,09
Basophils	0	0	0	0	0	0	0	0
Eosinophils	0,01	0,07	0,06	0,03	0,01	0,07	0,09	0,04
TSP (g/l)	48	55	63	61	72	67	67	68
GGPT (IU/l) 25 °C			14	16,5	14,8	27,14	21,14	30,5

(Day 200 – pups appeared to be clinically healthy and these values are taken as normal)

The skin condition deteriorated with the alopecia spreading to involve the head, legs, abdomen, dorsal sacral area and the tail. The papules remained restricted to the head.

With time, their appetites improved, the faeces became firmer and the pups became more playful.

Haematological and blood chemistry tests performed on Day 36 showed an improvement in the blood picture and plasma protein status.

On Day 37 the only female pup, which by now was smaller than the two males, was found to be acutely lame. Radiographic examination showed a fracture of the proximal epiphysis of the tibia and fibula and the leg was subsequently immobilized for twelve days. She was separated from her litter mates for three days during which period she ate considerably less and also seemed very listless. On returning the pup to her littermates, she assumed a submissive crouching position with ears flattened on the neck, whilst the two male-pups mockbit her in the neck and loins. This was accompanied by much twittering by all three pups. Following her return to her litter-mates, there was an immediate and noticeable improvement in the female pup's appetite and general habitus.

The pups now showed a gradual but steady improvement in their general condition. There was also a marked regrowth of normal hair on all the hairless patches. It was noted that the pups never defaecated in their sleeping area or nest. The intake of grass was also observed from time to time.

Fresh clean water was provided on a daily basis. The water was not only used for drinking but on occasions the animals were noticed to stand in it.

During the heat of the day the animals stayed mainly in the shade but always lying close to one another. On cooler days they could be found lying stretched out in the sun.

Up to the age of approximately six months these pups were caught fairly easily by cornering them and catching them by the nape of their necks while wearing protective leather gloves. As the pups became older and heavier, eventually weighting just over 17 kg when they were approximately 10 months old, this method became impossible and dangerous. Even the conventional noose was of no help when trying to catch three cornered and aggressive wild dog subadults. Whenever cornered or whenever threatened the three subadults would join to form a small but formidable pack. It became almost impossible to catch these pups in "their little pack". Separating the three pups facilitated matters. The aggressive interference of the others was in this way excluded and the female subadult in particular became almost submissive when separated. Protective gloves, a dog-catcher and a hessian bag were still however essential aids. Once the dog was snared, it was held down, the hessian was thrown over its head and it was grabbed by the nape by an assistant wearing protective leather gloves.

This method of capture often resulted in a lot of running and jumping and was always accompanied by a good deal of vocal communication between the subadults. The prescribed method eventually lead to the precipitation of stressful symptoms such as vomition on approach and defaecation when the dogs were released (to be reported elsewhere).

Results of special examinations

No helminth parasite ova could at any stage be demonstrated in the faeces. Microscopic examination of a blood drop demonstrated the presence of *Dipetalonema recondoitum* microfilaria.

Both the macroscopic post-mortem examination as well as the histopathological examination of various internal organs revealed nothing abnormal although there was some indication of an early enteritis. No pathogenic bacteria could be isolated from internal organs.

Examination of skin scrapings as well as cultivation of hairs were negative for mites, fungi and bacteria. Skin biopsies taken on Day 36 showed moderate to severe areas of hyperkeratosis but no other epidermal changes.

Examination of blood smears revealed the presence of *Hepatozoon canis* in one of the pups.

Results of haematological and blood chemistry tests are given in Table 2.

Radiological examination of the right femur of the female pup showed thinned but reasonably well calcified cortices. A pathological fracture of the proximal epiphyses of the tibia and fibula was evident.

DISCUSSION

These pups were caught in the wild, removed from the wild and tranported over a considerable distance to

highly artificial surroundings. Presumably they were still suckling. They were thus drastically deprived of (a) the maternal and social care of the pack; (b) their mother's milk and vomitus and (c) the microenvironment as created by the den and the mother's body within the den. These factors are of utmost importance to highly social animals such as wild dogs[2].

The pups were also subjected to handling and transport stress. Stressful stimuli and the inability of the pup to cope with them could have resulted in stimulation of the hypophyseal-adrenocortical axis with the subsequent elevation of cortico-steroid levels in the bloodstream. Sustained pituitary-adrenocortical arousal may result in diminished resistance to infection due to immunosuppression. Latent viral, bacterial, protozoal and infestations by internal parasites may become more severe in stressed animals[3]. Thus stress, plus the change in diet, could have triggered off pathogenicity of normal gut inhabitants resulting in gastro-intestinal disturbances.

Diet is *extremely* critical and the present attempt to raise wild dog pups could have been more successful if it would have been initially possible to feed a diet closer to the natural diet.

The exact cause of death in three pups remains unknown but it is believed that the diarrhoea was triggered off by stress and nutritional causes although no pathogenic bacteria could be isolated from the internal organs. It is possible that a virus could have been the aetiological agent.

Encke[1] reported mortality due to worm infestations despite the absence of demonstrable ova in the faeces. In these cases both faecal examinations and macroscopic examination of the gut were negative for helminth parasites.

Having survived the initial onslaught of acute diarrhoea the remaining three pups went through a period of low grade diarrhoea. The latter condition probably resulted in a malabsorbtion syndrome which was responsible for the low total serum protein levels, the low grade anaemia, the skin and bone condition as well as the generally poor physical condition.

Thus incorrect nutrition and malabsorbtion were probably responsible for the alopecia and papular dermatitis because no specific infectious aetiological agent could be isolated. The condition recovered spontaneously when the diarrhoea subsided and the appetite improved.

In the present attempt to raise wild dog pups it was felt that some of them were able to survive a very critical period due to the feeding of vomitus. The use of vomitus is advantageous in that it is partially digested, warm and very fluid in nature. This method has the disadvantage that "donor" dogs often develope a resistance to the effects of apomorphine and can thus usually be "used" only once or twice.

Daily handling or examination of each pup at close range is absolutely essential. It was originally decided not to handle the pups in an attempt to minimize taming. However from Day 7 onwards the pups were handled at least twice daily, without them becoming excessively tame.

The pups were bathed to improve the general hygiene of their coats. It was felt that they lacked the cleaning effect of natural social licking from adults in the pack.

The author would like to suggest that if wild dogs should be reared for any particular reason, this should be done as close to natural surroundings as is possible. Food items normally consumed by wild dogs should also be given preference.

To summarise the following points should be considered in the rearing of wild dogs:

1. minimize stress
2. stay as close to natural surroundings as possible
3. simulate the natural diet as far as possible
4. observe and handle daily
5. ensure adequate fluid intake
6. provide milk, fresh clean water and shelter
7. do not separate puppies – even the sick or injured should be kept close to littermates.

ACKNOWLEDGEMENTS

The author would like to thank the nursing and technical staff of the Department of Medicine for their invaluable assistance and the Director, Nature Conservation Division, Transvaal Provincial Administration for permission to undertake the project.

REFERENCES

1. Encke, W 1962 Hand-rearing cape hunging dogs *Lycoan pictus* at the Krefeld zoo. International Zoo Yearbook 4: 292–293
2. Estes D Richard, Goddard John 1967 Prey selection and hunting behaviour of the African Wild Dog. Journal of Wildlife Management Vol 31 No 1 p 52–70
3. Henry, James P 1976 Mechanisms of psychosomatic disease in animals. Advances in Veterinary Science and Comparative Medicine. V 20 p115–145

FORELEG LAMENESS IN RAPIDLY GROWING DOGS*

LEA STOGDALE

ABSTRACT: Stogdale L. **Foreleg Lameness in rapidly growing dogs.** *Journal of the South African Veterinary Association* (1979) **50**, No. 3, 193–200, (En) Department Medicine, Faculty Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Foreleg lameness caused by the interactions of diet and rapid growth rate is all too frequently encountered in the large and giant breeds of dogs. In this paper, the influence of rapid growth rate and growth hormone on bone formation is briefly considered. The important causes of this problem are discussed. These are hypertrophic osteodystrophy, osteodystrophy II, retained enchondral cartilage cores, panosteitis and nutritional secondary hyperparathyroidism. Rickets and hypertrophic pulmonary osteoarthropathy are also considered. Emphasis is placed on the aetiology, radiographic diagnosis and rational treatment. A case report of a 6-month-old Great Dane with osteodystrophy II and retained enchondral cartilage cores in the ulnar metaphyses is presented as an example of such a problem.

INTRODUCTION

Foreleg lameness is a commonly encountered problem in young, growing dogs of the large and giant breeds[2 3 10 17 20 22]. Apart from traumatic injuries, foreleg lamenesses are usually caused by the interactions of diet and rapid growth rate[2 3 10 18 22]. These lamenesses frequently present a diagnostic problem to the veterinary practitioner, but it is important that the correct diagnosis be established, and the aetiology recognized, so that the most suitable treatment can be instituted. Incorrect diagnosis and management may result in a permanent defect in the limbs of an affected dog[2 3 5 7 22 25]. This paper briefly considers the influence of rapid growth rate and growth hormone on bone formation. The aetiology, diagnosis, and rational treatment of the various diseases of growth and nutrition which affect the forelegs of the large and giant breeds of dogs are described. A case report of such a problem is presented as an example.

DISCUSSION

A number of skeletal abnormalities occur in the rapidly growing large breeds of dogs which lead to lameness[2 3 18 20 22]. These animals are particularly susceptible to locmotory problems because of their very rapid rate of growth[3 22]. This affects the entire skeleton but is frequently manifest initially in defective development of the distal radius and ulna, as these bone areas have a particularly rapid rate of growth[22]. The enthusiasm of owners to supplement the dog's generally excellent quality and generous diet, with minerals and vitamins increases the incidence of abnormalities[2 8 1]. Additionally, the immature bones have to support a large body mass and to withstand the mechanical traumas imposed by the normal activity of the young dog[18]. It would appear from the high frequency of problems which occur, with only minor predisposing causes, that the skeletal metabolic status of these large dogs is very close to homeostatic imbalance[22].

In immature animals, a number of hormones exert a profound influence on the growth plate cartilage. The anabolic pathways and growth of epiphyseal cartilage are stimulated by growth hormone (somatotropin) in particular, and by the thyroid hormones and androgens, to a lesser extent. The process of cartilage maturation and replacement by bone tissue is stimulated by the thyroid hormones and the sex hormones[11 12]. In the large and giant breeds of dogs, growth hormone levels are raised above those of small and medium sized dogs[13]. During the first 6 to 7 months of life, 90% of the growth in length of bones takes place, with the most rapid growth and ossification period occurring between 4 and 5 months of age[22]. Most radial and ulnar deformities in dogs occur in this age group[5]. The remaining 10% of growth in length of bones and the process of gradual bone maturation takes place from the age of 7 months to between 10 and 13 months, at which time the epiphyseal plates close[18 22]. This latter process is influenced by the thyroid hormones and the gradual rise in androgen and oestrogen levels which occur with sexual maturity[6 11]. Selection pressure for size in the giant breeds of dogs is also selection of growth hormone levels[13]. It would appear that in some of these dogs, the normal endocrinological balance with respect to the growth and maturation of bones is overridden by an excess of growth hormone. This predisposes to bone abnormalities.

The number and subtle differences of front leg abnormalities which occur in the large and giant breeds of dogs frequently leads to confusion[2 10 16 17 20]. An accurate diagnosis is essential and requires good quality radiographs (Table 1). Then, a rational decision as to aetiology, prognosis and treatment may be made. A short description of the relevant and important foreleg conditions, which may occur in rapidly growing dogs, follows.

Hypertrophic osteodystrophy is the usual name given to the condition in which excessive new bone is deposited in the soft tissue surrounding the distal radial, ulnar and tibial metaphyses[1 2 18 20 22]. The condition occurs in rapidly growing dogs which are fed high levels of minerals and irradiated vitamin D[7 20 22]. This causes excessive stimulation of bone metabolism[20]. Affected dogs are reluctant to move and show intermittent symptoms of pyrexia, anorexia and depression. The distal metaphyses of the affected bones are swollen, hot and painful[1 7 18 20 22]. Radiographically (Fig. 1), the affected metaphyses are enlarged and have an increased density, but contain radiolucent areas. As the disease progresses, bone is laid down outside the periosteum. This cuff of bone, of lace-like appearance, extends from the metaphyseal region proximally to surround the distal portion of the diaphysis. It is clearly separated from the bone by soft tissue. The skeleton is of normal density, and the epiphyses and growth plates are normal[1 2 3 7 14 20 22]. Soft tissue metastatic calcification occurs in severe cases. The prognosis is favourable when the diet is corrected. The symptoms disappear and the excess bone is gradually resorbed[1 22]. Retardation of the growth of

*(Corrected version of article originally published Vol. 50, No. 2 pages 61–68)

0038-2809/79/03/0193–0200

Table 1: DIFFERENTIAL DIAGNOSIS OF FORELEG LAMENESS IN RAPIDLY GROWING DOGS

Disease Condition	Systemic symptoms	Foreleg symptoms	Bones affected	Radiographic findings				
				Skeletal density	Epi-physis	Growth plate	Metaphysis	Diaphysis
Hypertrophic osteo-dystrophy	inactivity; intermittent depression, pyrexia and anorexia	lameness; carpal swelling, heat and pain	bilateral; radius ulna ± tibia	N	N	N	enlarged; ↑ density; radiolucent band parallel to growth plate; cuff of bone outside the periosteum	cuff of bone outside the periosteum, extending from the metaphysis
Osteo-dystrophy II	N	lameness; carpal en-largement	bilateral; radius ulna	N	N	N	enlarged; gra-nular radio-lucent areas	N
Retained enchondral cartilage cores	N	external rota-tion of carpi; lateral devia-tion of paws; cranialward bo-wing of radius	bilateral; ulna	N	N	± distor-tion	radiolucent wedge exten-ding proxi-mally from the growth plate	radiolucent wedge exten-ding from the metaphysis; radial bowing cranialward
Panosteitis	shifting lameness	lame in a leg for 1 to a few weeks. Then another leg affected	any long bone	N	N	N	N	patchy densi-ties and ↓ detail in medullary cavity; ±periosteal new bone formation
Nutritional secondary hyperpara-thyroidism	N ± vertebral compression ± pelvic collapse	Lameness and pain from pathological fractures	bilateral; all bones	↓	N shape ↓ density	N	N shape ↓ density	± pathological fractures
Rickets	Slow growth rate; muscle weakness; ↓ activity; costochondral junctions en-larged	lameness and pain from pathological fractures; epiphyseal en-largement	bilateral; all bones	↓	↓ density	widened irregular edges	↓ density widened irre-gular edges	bowing of long bones; ± folding fractures

N normal; ↑ increase; ↓ decrease; ± occurs sometimes.

the distal ulnar epiphyseal plate occurs in severe cases of long standing[3 15].

Osteodystrophy II is considered to be either a variant form of hypertrophic osteodystrophy or a separate disease entity. The condition occurs in the large and giant breeds of dogs aged 7 months or less, which are fed a balanced diet, *ad lib.* The aetiology is unknown but is probably associated with a very rapid rate of bone growth[20]. The clinical characteristics are lameness and enlargement of the carpi with an abscence of systemic symptoms. The abnormalities are generally restricted to the distal radius and ulna. Both front legs are affected but to varying extents. Radiographically (Fig. 2), the metaphyses are enlarged and areas just proximal to the growth plates have a groundglass or granular appearance. Radiolucent areas or a band may be seen in the metaphyses. The diaphyses, growth plates and epiphyses are normal. The irregular appearance of the metaphyseal region is attributed to disintegration or structural disorganization of the primary trabeculae where enchondral osteogenesis is most active. This disintegration and degeneration seems to be caused by an excessive rate of growth resulting in defective mineralization[20]. If follow-up radiographs are taken, after clinical symptoms have subsided, a cuff of new bone surrounding the distal metaphysis is seen in some of these cases. This suggests that osteodystrophy II is merely a milder or more chronic form of hypertrophic osteodystrophy[19]. Affected dogs become sound without any specific therapy. A moderate level of exercise and a balanced diet result in the gradual regression of the bony abnormalities.

Retained enchondral cartilage cores is a distinct pathological condition affecting the metaphyseal region of the ulnar bones. It occurs frequently in the giant breeds of dogs[3], generally occurring between the ages of 4 and 7 months of age. The aetiology is postulated as being an insufficient blood supply to the distal ulnar metaphyses[22].

The appendicular skeleton is formed by endochondral ossification. Initially, cartilage is laid down and becomes mineralized by osteoblasts which emigrate from the perichondrium or periosteum. Blood vessels grow into the calcifying cartilage and the chondrocytes are resorbed, leaving cavities. These are occupied by osteoblasts which then proceed to form bone on

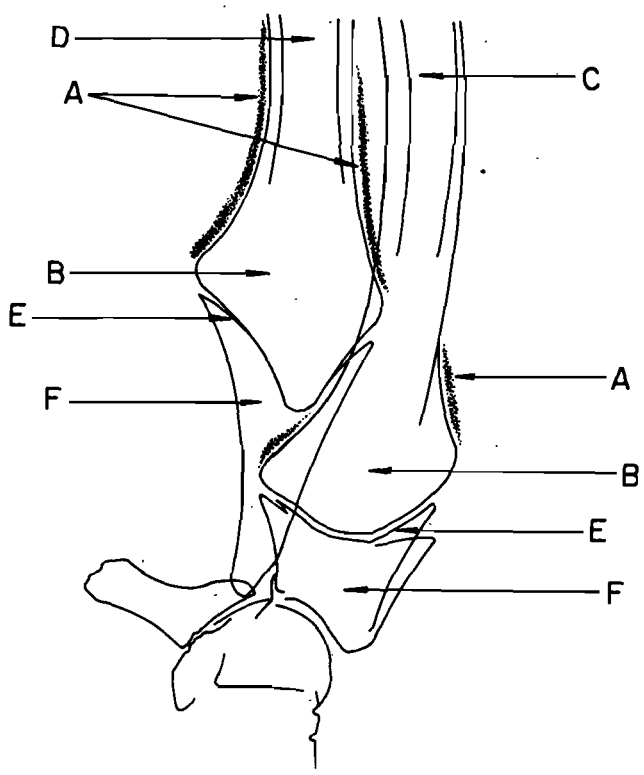


Fig. 1. Diagram of the lateral radiograph of the carpal findings in hypertrophic osteodystrophy of moderate severity. Extensive periosteal new bone formation extends from the metaphyses of both the radius and ulna, to surround the distal shafts of the bones (A). These cuffs of lace-like bone are separated from the bones by soft tissue. The metaphyses are enlarged and have an increased density with areas of radiolucency (B). Normal appearing diaphysis of the radius (C), diaphysis of the ulna (D), growth plates (E), and epiphyses (F).

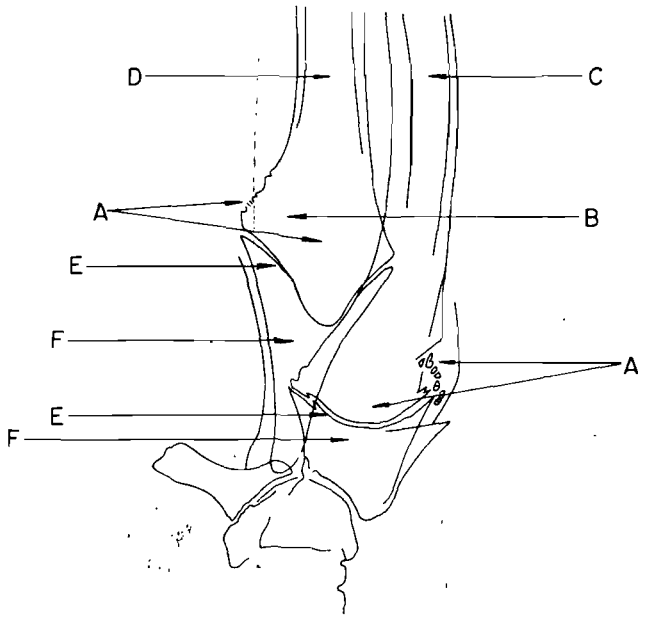


Fig. 2. Diagram of the lateral radiograph of the carpal findings in osteodystrophy II. Enlargement of the metaphyses resulting in flaring, and, the uneven granular appearance of the disorganized trabeculae causing cortical irregularity (A). Radiolucent areas in the metaphysis (B). Diaphysis of the radius bowed cranialward (C). Normal diaphysis of the ulna (D), normal growth plates (E), and normal epiphyses (F).

the mineralized trabeculae[16 23]. If the blood supply to the area is inadequate, the cartilage matrix does not become mineralized and chondrocytes are not resorbed[26]. When this occurs, cartilage is not replaced by bone[18]. Three factors contribute to causing the inadequate blood supply to the central area of the distal ulna. Firstly, the nutrient artery, which supplies this region, enters the ulna near to the proximal end of the bone and is directed dorsally, away from the distal growth plate[18 22]. Secondly, the distal growth plate of the ulna is extremely active, accounting for 85% of the growth of that bone[5 22]. Thirdly, at the time of greatest enchondral ossification, which occurs at 4½ to 5 months of age, the width of the ulna is approximately twice that of the ulna of the adult dog[22]. It would appear that the extremely rapid growth of the distal ulna in well fed giant breeds of dogs can result in an inadequate blood supply to the central region of the bone and retention of the enchondral cartilage cores. The growth rate of the distal ulnar growth plate is decreased by the inadequate blood supply and the presence of the retained cartilage core. The proximal ulnar epiphysis is unaffected but is very slow growing, normally accounting for only 15% of the final length of the bone, namely the olecranon[5 18 22]. The radius continues to lengthen normally and so a disparity occurs between these interdependent bones[5 16]. The strong interosseus ligament, and the articulation of both the radius and the ulna with the humerus, allows little movement of one bone relative to the other, proximally. Distally, the radius lies craniomedially and articulates principally with the intermedioradial carpal bone[23]. As the radius lengthens relative to the ulna, the carpus is rotated outwards, the paw is deviated laterally and the radius develops a cranialward bow[3 18 22].

The symptoms occurring with retained enchondral cartilage cores are outward rotation of the carpi, lateral deviation of the feet (valgus deformation) and cranialward bowing of the radius[3 15 18 22]. This triad is seen in both front legs but the degree may vary. The presence of cartilagenous cores is diagnosed when good quality radiographs are taken of the carpal area. Radiographically (Fig. 3), a core appears as a radiolucent strip extending dorsally from the growth plate, through the metaphyseal region into the diaphysis[3 14 22]. The width may reach 8 mm and the length can be up to 4 cm. The cartilage adjacent to the core is ossified normally. The growth plate may be distorted but the epiphysis is normal[22].

From the age of 7 to 10 months the growth rate of the long bones is fairly slow. During this time the width of the ulna decreases and the ulnar growth plates and metaphyses receive sufficient blood, enabling normal enchondral ossification and growth to occur[18]. The cartilage cores gradually disappear and the ulnae appear normal on radiographs. The aim of treatment is to decrease the growth rate of the dog in order to allow the blood supply of the ulna to catch up with its requirements. This is accomplished by limiting the amount of food given to the dog[22]. For large dogs, 50 g/kg/day of good quality, balanced food is recommended[9]. The growth plates remain open until 10 to 13 months of age. This usually provides sufficient time for the condition to be corrected[22]. Once the growth plates have closed, any defect in the conformation of the legs will be permanent. If this occurs corrective osteotomy is required to straighten the leg[3 5 15].

Panosteitis affects the long bones of young growing

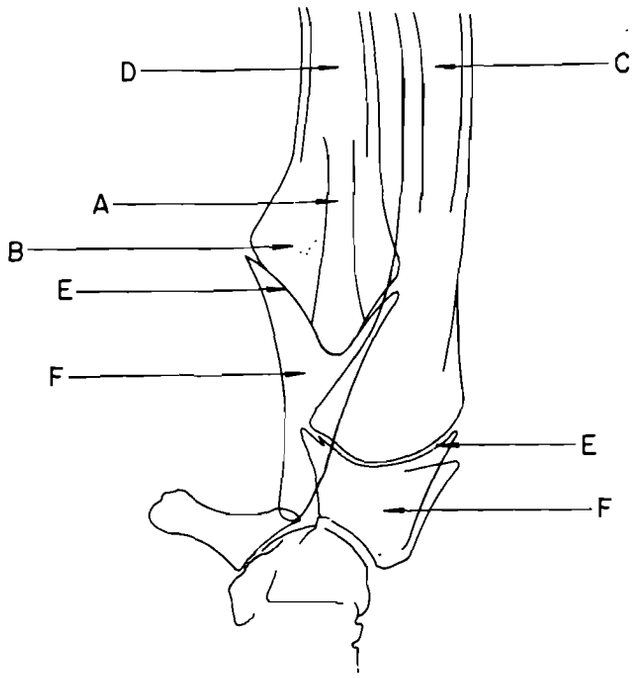


Fig. 3. Diagram of the lateral radiograph of the carpal findings in retained enchondral cartilage cores. Radiolucent (dark on a radiograph) strip extending from the growth plate, through the metaphysis, into the shaft of the ulna (A). The ulnar metaphysis is of normal size for a growing dog and has an uniform radiopaque (white on a radiograph) appearance (B). Diaphysis of the radius bowed cranialward (C). Normal diaphysis of the ulna (D), normal growth plates (E), and normal epiphyses (F).

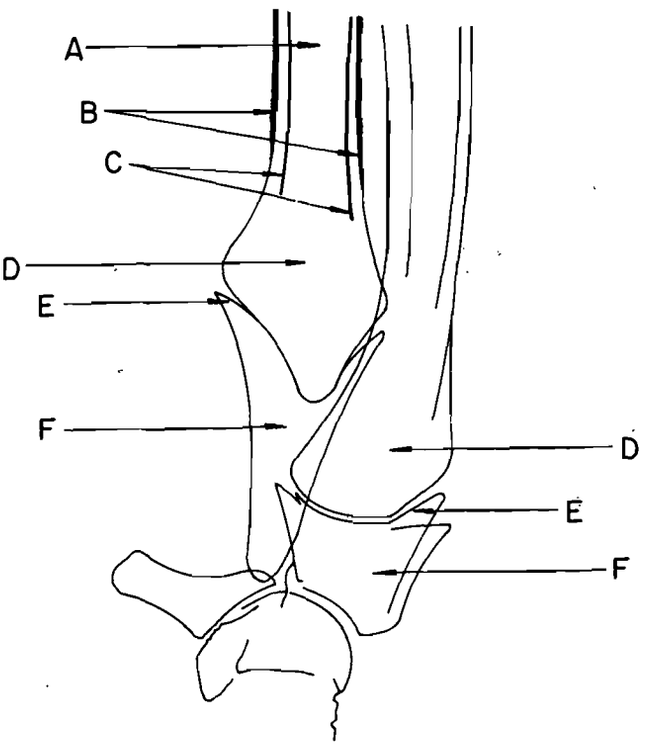


Fig. 4. Diagram of the lateral radiograph of the carpal findings in the middle phase of panosteitis. Diaphysis of the ulna has a patchy increase in density of the medullary cavity (A). There is also periosteal (B) and endosteal (C) new bone formation which results in accentuation of these lines. Normal metaphyses (D), normal growth plates (E), and normal epiphyses (F).

dogs. The dogs are most commonly of the large breeds, with German Shepherds and males predominating. They are usually between 5 and 12 months of age[3 4]. The aetiology is unknown. Excessive osteoblastic and fibroblastic activity occurs in the periosteum, endosteum and medullary cavity[4]. Clinically, the condition manifests as a shifting lameness. The acute pain persists in any one leg from 1 to a few weeks. Another leg may then be affected. There are no systemic symptoms. Firm palpation of the shafts of the affected bones causes a pain response. Good quality radiographs are essential for a diagnosis. Radiographically (Fig. 4), there is an initial increase in density and a decrease in detail in the medullary area of the affected bone. This progresses to patchy densities in the medulla, and periosteal new bone formation in severe cases. After 4 to 6 weeks, the densities regress leaving a trabecular pattern which is coarser than normal[3 4]. This condition is self-limiting so symptomatic therapy, in the form of analgesics, is the only therapy indicated[4].

Nutritional secondary hyperparathyroidism occurs in young dogs and cats which are fed food deficient in calcium and/or high in phosphorus, such as a predominantly meat diet. This results in calcium deficiency. The calcium deficiency stimulates parathyroid hormone secretion which causes calcium and phosphate mobilization from throughout the skeleton. Inadequate mineralization of the skeleton (osteomalacia) occurs, and the bones become weakened. Bowing of the long bones and pathological fractures are a frequent sequelae. The vertebral bodies may suffer compression fractures, and pelvic narrowing or collapse can occur[2 3 14 20]. Radiographically (Fig. 5), there is a generalized decrease in density (insufficient mineralization) and pathological fractures occur at points of greatest pressure. The epiphyses and metaphyses have a normal conformation (contrast rickets). Correction of the diet, along with supplementation of calcium, results in remineralization of the skeleton. Light splints, bandages, or casts are successful in correcting leg deformities caused by fractures[3].

Rickets is caused by an inadequate plasma level of active vitamin D (1,25 dihydroxycholecalciferol) along with a diet deficient in calcium and phosphorus. As dogs do not require exogenous vitamin D, rickets only occurs when a dog is deprived of sunlight for a few months[3 20]. Therefore, this condition is extremely rare[2 11 14 18 20]. Inadequate vitamin D results in generalized defective bone formation (osteoporosis) and decreased bone mineralization (osteomalacia).

Affected animals have a slow growth rate, muscle weakness, decreased activity and bone distortions. The carpal and tarsal joints, and the costochondral junctions become noticeably enlarged[20]. Radiographic changes (Fig. 6) include decreased density of all bones. The distal epiphyseal plates and metaphyses of the radius and ulna are radiolucent and have abnormally wide and irregular edges. The long bones are bowed and folding fractures are common[2 13 20]. Early cases respond well to vitamin D supplementation and dietary correction[2]. Severe cases are often left with permanent bone deformities.

A number of other conditions occur which cause foreleg lameness in large breeds of dogs. These diseases are

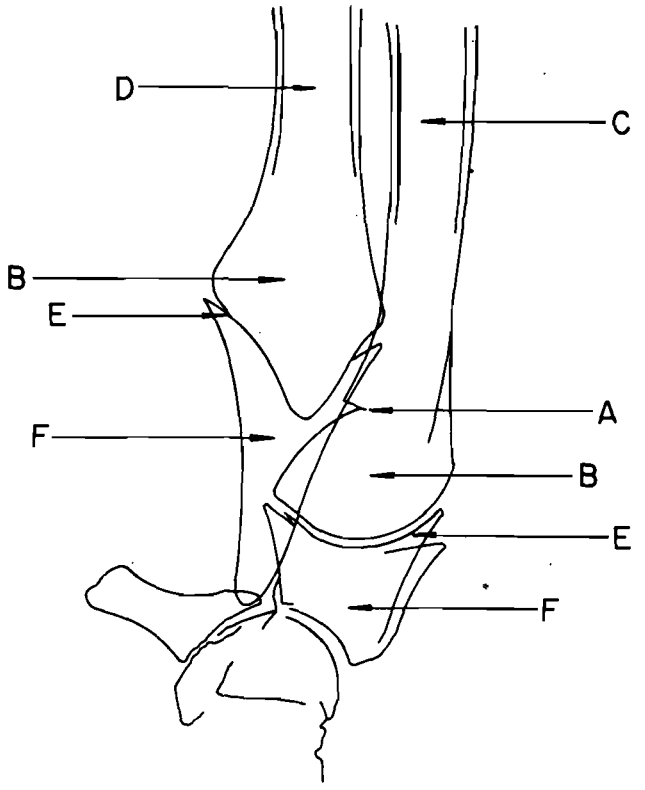


Fig. 5. Diagram of the lateral radiograph of the carpal findings in nutritional secondary hyperparathyroidism. Pathological folding fracture in the distal radial metaphysis (A). Demineralized metaphyses (B). The diaphyses of the radius (C), and the ulna (D) have very thin cortices and are decreased in density. Normal growth plates (E), and epiphyses (F).

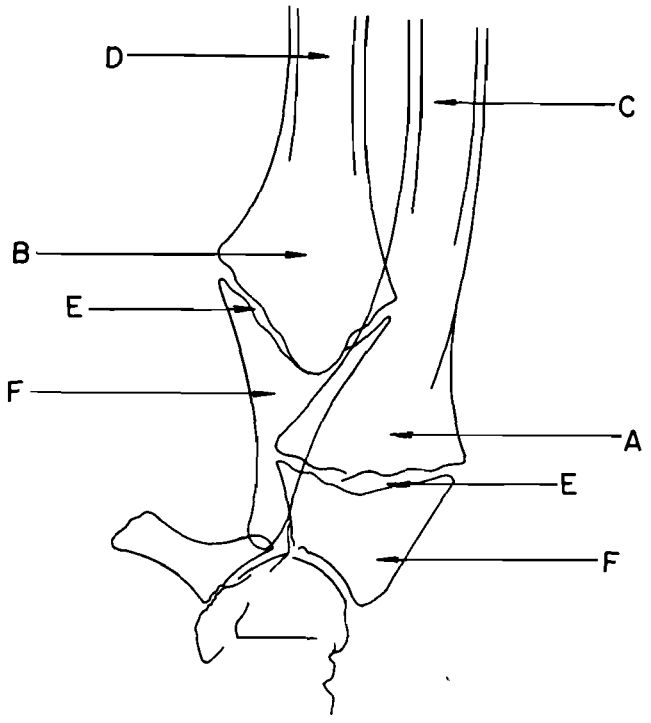


Fig. 6. Diagram of the lateral radiograph of the carpal findings in rickets. Radial (A) and ulnar (B) metaphyses are radiolucent and markedly enlarged, especially adjacent to the epiphyseal lines. The radial diaphyses is inadequately mineralized and is bowed cranialward (C). The diaphysis of the ulna is radiolucent (D). The growth plates are widened and have irregular metaphyseal and epiphyseal borders (E). The demineralized epiphyses appear radiolucent (F).

not necessarily associated with rapid growth or nutritional imbalance. Some cause systemic symptoms, but all depend on radiography for definitive diagnosis.

Hypertrophic pulmonary osteoarthropathy is almost invariably associated with a space-occupying lesion in the thoracic cavity[3 10 14]. The aetiology of the bone pathology is not well understood, but an autonomic neurovascular reflex is postulated[3]. Systemic symptoms vary with the nature of the thoracic lesion which is most commonly a neoplastic process. The carpal and antibrachial foreleg regions of both front legs are swollen, hot and painful. The metatarsal regions of the hindlegs may also be involved. Radiographic examination of the forelegs, (Fig. 7), reveals bilateral symmetrical periosteal new bone formation on the phalanges and metacarpal bones. This sometimes extends to the radius, ulna and tibia, and occasionally to the humerus and femur[10]. The primary lesion(s) can usually be observed on thoracic radiographs. Generally the prognosis is very poor due to the neoplastic nature of the lung mass. A few cases have responded to successful treatment of the thoracic pathology[10 14].

Ulnar growth plate injury due to trauma results in a decreased rate of growth of the bone, or may even cause premature closure of the distal epiphyseal plate[3 5 15 16 17 24 25]. The radius continues to grow at a normal rate. This results in cranial and medial bowing of the radius with abduction of the paw[1 3 15 16 22 24 25]. Only surgical correction is successful in realigning the leg. Either stapling the distal radial growth plate or osteotomy are used[5 15 25]. Lack of strength of the palmar carpal liga-

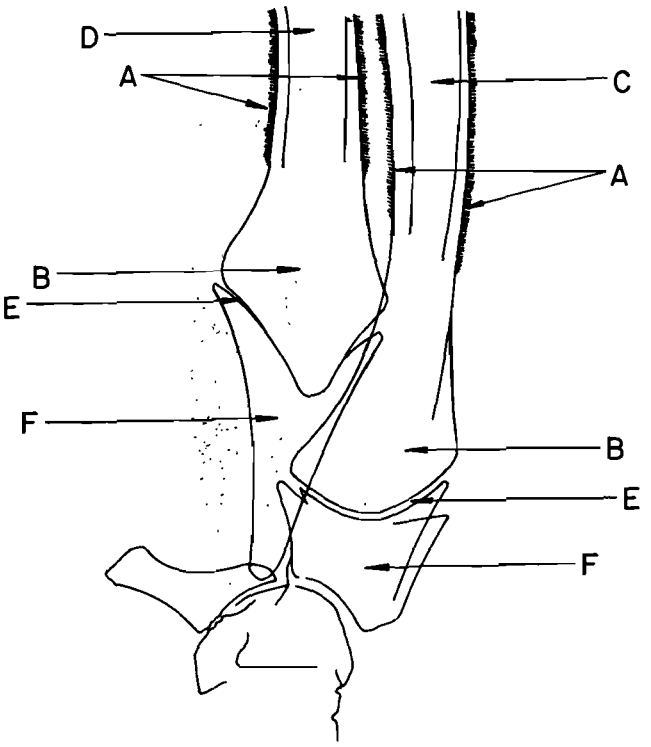


Fig. 7. Diagram of the lateral radiograph of the carpal findings in advanced hypertrophic pulmonary osteoarthropathy. Periosteal new bone formation occurs on the shafts of bones (A). The metaphyses appear normal (B). Radial (C) and ulnar (D) diaphyses are normally mineralized but have periosteal proliferations along their surfaces. The growth plates (E) and epiphyses (F) are normal.

ments is a commonly seen abnormality. Stretching of the ligaments results in overextension of the carpal joints. This is associated with vitamin, mineral or protein deficiencies. Overextension of the carpal joints is frequently seen in association with bone abnormalities. It is successfully treated by correcting the dog's nutrition[22].

Other bone diseases which must be considered when a unilateral foreleg lameness occurs, includes traumatic bone injury, osteomyelitis or osteitis, Brodey's abscess, osteochondromatosis, neoplasia and myositis ossificans. Traumatic injuries resulting in fracture, dislocation or collapse can be predisposed to by inadequate vitamins or minerals in the diet resulting in inadequate mineralization[22]. Joint conditions which must be differentiated include traumatic arthrosis, bacterial arthritis, rheumatoid arthritis, systemic lupus erythematosis polyarthritis, elbow dysplasia (ununited anconeal process) and osteochondritis dissecans.

CASE REPORT

A 6-month-old male Great Dane weighing 33 kg was referred to the Department of Veterinary Medicine with a history of intermittent lameness and lateral deviation of the left foreleg. Since the age of 3 months the dog had been fed a proprietary dog food *ad lib;* no mineral or vitamin supplementation had been given.

Significant clinical findings were restricted to the locomotory system. The distal metaphyses of the radius and ulna of both forelegs were enlarged. There was no soft tissue swelling, local hyperthermia, or pain on palpation of the front limbs. There was a valgus deformity of the left carpus resulting in abduction of the paw. The right fore limb was similarly malaligned but to a lesser degree. The radii were bowed cranialward. Upon exercise the dog was energetic and moved around with no sign of lameness. Faecal analysis, urinanalysis, haematology, serum enzymes, electrolyte and electrophoretic values were all within the normal range.

Upon admission, dorsopalmar and mediolateral radiographs were taken of both carpal joints (Figs. 8 and 9). The distal metaphyses of the radius and ulna of both forelegs were flared and had an irregular groundglass appearance. Cortical irregularities were evident on the cranial and medial aspects of the distal radial metaphyses (Fig. 8). Both of the distal ulnar metaphyses exhibited cortical irregularity on the caudal edge, a radiolucent band and a retained enchondral cartilage core. The radii were bowed cranialward (Fig. 9). All these findings were more marked in the left leg as compared with the right one.

The final diagnosis was osteodystrophy II with retained enchondral cartilage cores in the ulnar metaphyses. The aetiology was attributed to the rapid growth rate of the 6-month-old Great Dane which occurred with *ad lib* feeding. The only treatment instituted was a restriction of diet. The owner was advised to feed 50 g/kg, dry weight, of proprietary dog food daily. Radiographic examination was repeated three weeks after the initial examination (Fig. 10). When

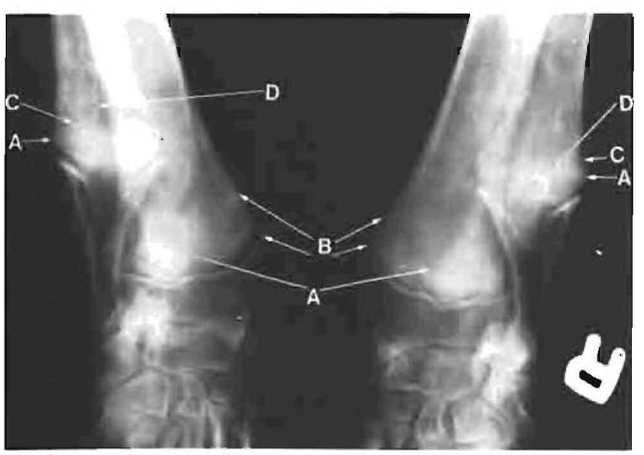


Fig. 8. Dorsopalmar radiograph of the left and right (R) carpal joints. In both forelegs the distal metaphyses of the radius and ulna are slightly flared and have an irregular groundglass appearance (A). Irregularity of the cortex is present in the medial aspect of the distal radial metaphyses (B). A radiolucent band extends across each of the distal ulnar metaphyses (C). A retained enchondral cartilage core is evident in both of the distal ulnar metaphyses and extends into the diaphyses (D).

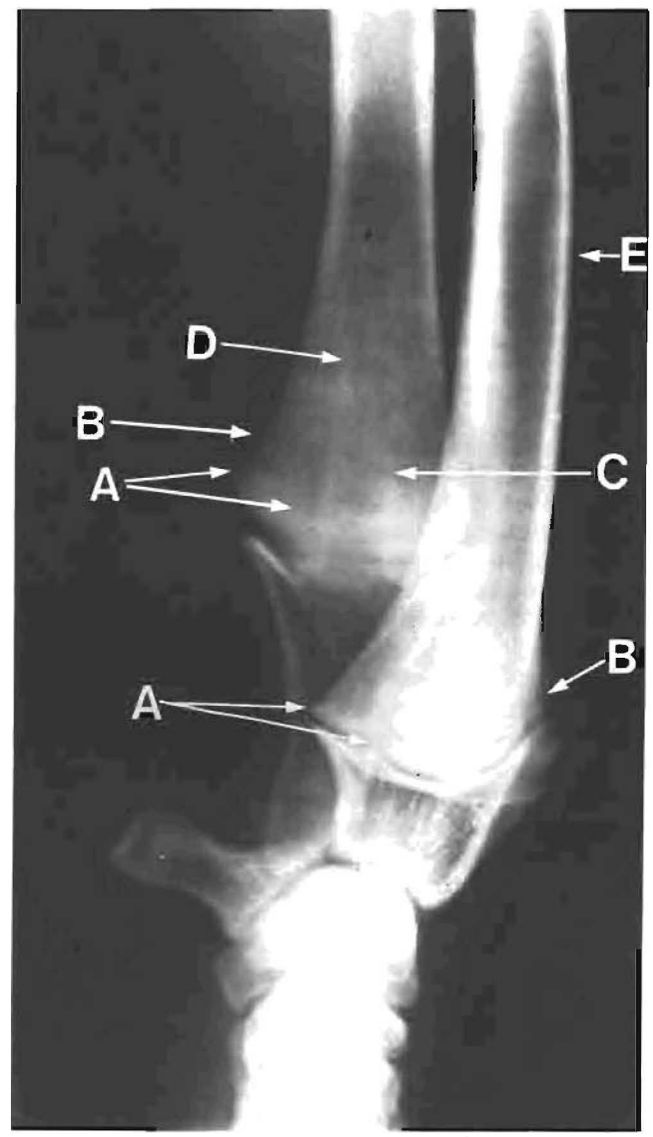


Fig. 9. Lateral radiograph of the left carpus. The distal metaphyses of the radius and ulna have an irregular groundglass appearance and are distinctly flared (A). Cortical irregularities are evident on the cranial border of the radial metaphysis and the caudal edge of the ulnar metaphysis (B). A radiolucent band extends across the metaphysis of the ulna (C). A retained enchondral cartilage core is seen in the ulna as a radiolucent wedge extending from the growth plate into the metaphysis and diaphysis (D). The radius is bowed cranialward (E).

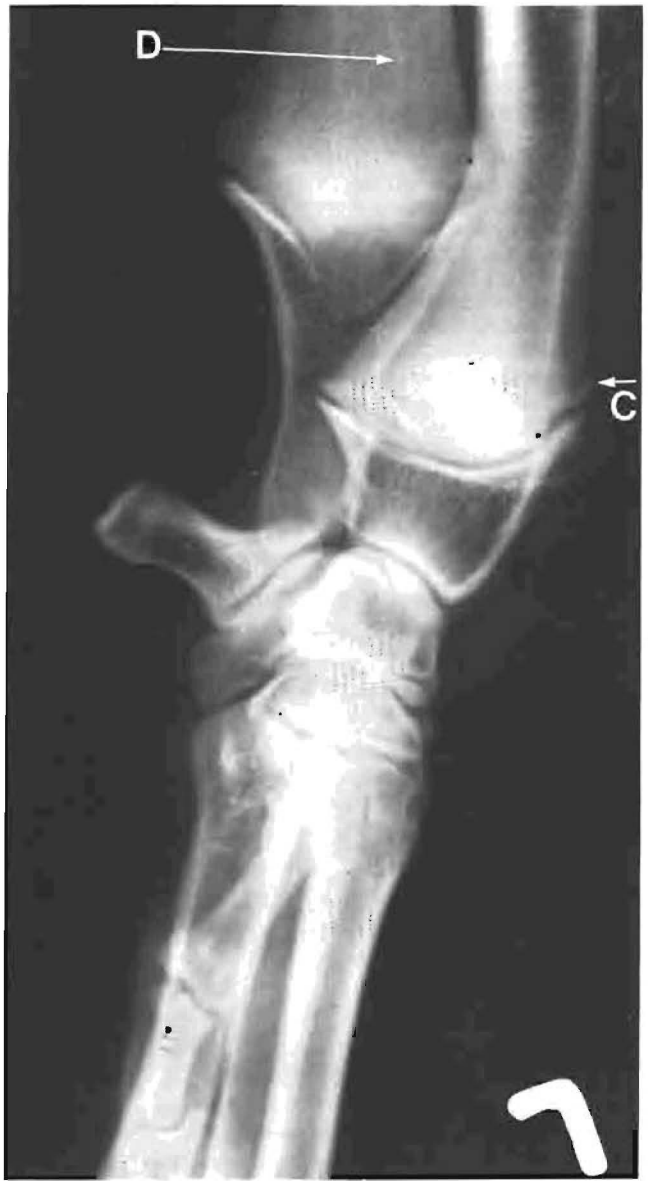


Fig. 10. Lateral radiograph of the left carpus taken 3 weeks after the initial examination. The irregularity of the radial metaphyseal cortex is less pronounced and has a smoother appearance (C). The enchondral cartilage core in the ulna is showing an increase in density (D).

compared with the initial radiographs, the cortical irregularities of the distal metaphyses were less pronounced and had a smoother appearance. The enchondral cartilage cores showed an increase in density. As the dog continued to grow the carpal enlargement slowly disappeared and the lateral deviation and abduction of the fore feet straightened. No lameness or joint pain recurred and 8 weeks after the restricted diet was commenced, the dog's conformation and carpal radiographs were normal.

CONCLUSIONS

The large and giant breeds of dogs have been continually selected for size, conformation and activity. This has resulted in a physiologically high level of circulating growth hormone and an extremely rapid growth rate of the skeleton. In addition, owners often feed these animals unbalanced diets which are either deficient or excessive in vitamins and minerals. The rapid growth rate alone, or in combination with a dietary imbalance, frequently results in disturbances in the circulation, metabolism and structure of the bones. Due to the high level of activity of the distal ulnar and radial metaphyses, this region is usually affected first and more severly than other parts of the skeleton.

The most frequently encountered abnormalities are hypertrophic osteodystrophy, osteodystrophy II, retained enchondral cartilage cores, panosteitis, ulnar growth plate injuries, and stretching of the palmar carpal ligaments. The diagnosis of these conditions depends on radiographic examination. The treatment is correction of the dietary imbalance and a slight slowing of the growth rate by feed restriction. This allows pathological growth to be converted to physiological growth which usually results in complete correction of the structural abnormalities.

ACKNOWLEDGEMENTS

Grateful thanks are extended to Dr D F Steyn for making the initial diagnosis and referring the case described in this report, to Professor C J Roos, Professor J M W Le Roux and Dr L Bomzon for reviewing the original manuscript, and Mrs J Oberholzer for doing the typing. The author is indebted to the staff of the Photographic unit, Department of Medicine, University of the Witwatersrand Medical School, for transcribing and photographing all the line diagrams used in this paper.

REFERENCES

1. Alexander J W 1978 Hypertrophic Osteodystrophy. Canine Practice 5: 48
2. Bennett D 1976 Nutrition and bone disease in the dog and cat. Veterinary Record 98: 313
3. Brown S G 1975 Skeletal disease in: Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat, Edited by Ettinger S J, W B Saunders Company, Philadelphia
4. Böhning R H, Suter P F, Hohn R B, Marshall J 1970 Clinical and Radiologic Survey of Canine Panosteitis. Journal of the American Veterinary Medical Association 156: 870
5. Denny H R 1976 The Treatment of Growth Disturbances of the Canine Radius and Ulna.Veterinary Annual 16: 170
6. Faulkner L C 1969 Male reproduction in: Veterinary Endocrinology and Reproduction, Edited by Macdonald L E. Lea & Febiger, Philadelphia
7. Grøndalen J 1976 Metaphyseal osteopathy (hypertrophic osteodystrophy) in growing dogs. A clinical study. Journal of Small Animal Practice 17: 721
8. Hedhammer A, Fu-Mung W U, Krook L, Schryver H F, de Lahunta S, Whalen J P, Kallfelz F A, Nunex E A, Hintz H F, Sheffy B E, Ryan G D 1974 Overnutrition and Skeletal Disease. The Cornell Veterinarian 64: 9
9. Kirk R W 1974 Estimated daily food intakes required by dogs of various sizes in: Current Veterinary Therapy V Small Animal Practice, Edited by Kirk R W. W B Saunders Company, Philadelphia
10. Knecht C D 1975 Diseases of Bone and Diseases affecting Bone. Archives 4: 46
11. Krook L 1971 Metabolic Bone Diseases in Dogs and Cats. Proceedings of the 38th Annual General Meeting of the American Animal Hospital Associaton 38: 350
12. Lebovitz H E, Eisenbarth G S 1975 Hormonal Regulation of Cartilage Growth and Metabolism. Vitamins and Hormones: Advances in Research and Applications 33: 575
13. McDonald L E 1969 The pituitary gland in: Veterinary endocrinology and Reproduction. Edited by McDonald L E Lea & Febiger, Philadelphia
14. Morgan J P 1972 Radiology in Veterinary Orthopedics. Lea & Febiger, Philadelphia
15. Newton C D 1974 Surgical Management of Distal Ulnar Physeal Growth Disturbances in dogs. Journal of the American Veterinary Medical Association 164: 479
16. O'Brien T R 1971 Developmental Deformities due to Arrested Epiphyseal Growth. Veterinary clinics of North America 1: 441

17. O'Brien T R, Morgan J P, Suter P F 1971 Epiphyseal Plate Injury in the Dog: a Radiographic Study of Growth Disturbances in the Forelimb. Journal of Small Animal Practice 12: 19
18. Parkes L J, Riser W H, Martin, 1966 Clinico-Pathologic Conference. Journal of the American Veterinary Medical Association 149: 1086
19. Rendano V T, Dueland R, Sifferman R L 1977 Metaphyseal Osteopathy (Hypertrophic Osteodystrophy). Journal of Small Animal Practice 18: 679
20. Riser W H 1964 Radiographic Differential Diagnosis of Skeletal Diseases of Young Dogs. Journal of the American Radiology Society V: 15
21. Riser W H, Parkes L J, Rhodes W H, Shirer J F 1969 *Genu Valgum:* A Stifle Deformity of Giant Dogs. Journal of the American Radiology Society X: 28
22. Riser W H, Shirer J F 1965 Normal and Abnormal growth of the Distal Foreleg in Large and Giant Dogs. Journal of the American Radiology Society VI: 50
23. Sissons S, Grossman J D 1953 Osteology in: The Anatomy of the Domestic Animals, 4th edn. W B Saunders Company, Philadelphia
24. Skaggs S, DeAngelis M P, Rosen H 1973 Deformities Due to Premature Closure of the Distal Ulna in Fourteen Dogs: a Radiographic Evaluation. Journal of the American Animal Hospital Association 9: 496
25. Vaughan L C 1976 Growth Plate Defects in Dogs. Veterinary Record 98: 185
26. Yabsley R H, Harris W R 1965 The Effects of Shaft Fractures and Periosteal Stripping on the Vascular Supply to Epiphyseal Plates. Journal of Bone and Joint Surgery 47A: 551

CANINE·THORACOLUMBAR DISC DISEASE

G E FROST

ABSTRACT: Fost G E **Canine thoracolumbar disc disease.** *Journal of the South African Veterinary Association* (1979) **50** No. 3 201–204 (En) Practitioner, Box 50258, Randburg 2125, Rep. of South Africa.

The incidence, pathology and accurate localisation of canine intervertebral disc lesions are reviewed and the surgical technique for wide dorsal laminectomy is described.

INTRODUCTION

Intevertebral disc protrusion or extrusion occurs in all breeds of dogs. The condition is most common in the chondrodystrophic breeds and can occur at an early age. In these animals this is related to the early degeneration of the intervertebral discs.

Breed incidence

Statistically the following breeds suffer most frequently from disc problems, in order of incidence.

1. Dachshund
2. Pekingese
3. Miniature Poodle
4. Beagle
5. Miniature Schnauzer
6. Cocker spaniel
7. Corgi

Site incidence

A recent analysis of 187 cases of thoraco-lumbar disc disease undertaken by workers at the Animal Medical Centre in New York showed the order of frequency of disc rupture at each interspace to be as follows:

1. T12–13
2. T13–L1
3. L1–2
4. T11–12
5. L2–3
6. L3–4
7. L4–5
8. T10–11
9. 56

This corresponds closely with other studies which have shown that ± 80 % of intervertebral disc protrusions occur between T11 and L3(5).

Pathology

The occurrence of this neurologic syndrome at three years of age is not uncommon and may even occur at two years of age. In a dog less than one year old, however, thoraco-lumbar signs are usually due to other causes. The condition is seen in non-chondrodystrophic breeds usually at a later age (5–12 years) and in these cases the condition is often termed geriatric disc disease.

In the chondrodystrophic breed, the disc degeneration is essentially a chondroid metaplasia with dehydration, necrosis and calcification of the nucleus pulposus. Degeneration of the *anulus fibrosus* occurs at the same time. The nucleus loses its normal hydrodynamic shock absorbing ability and becomes predisposed to protrusion or extrusion. The degenerated *anulus,* especially the narrower dorsal part, easily disrupts under even minimal stress and a disc prolapse occurs.

The dynamic force with which the disc becomes extruded, the inflammatory reaction in the epidural space, and the specific location of the extrusion with respect to the diameter of the vertebral canal, are important factors determining the severity of the cord lesion. Not only is the size of the protrusion important but also the rate at which it occurs, as will be further illustrated later. A small, rapidly occuring or explosive protrusion will usually result in a much more severe neurologic dysfunction, than will a large slowly progressive protrusion.

Pathology

The extrusion can be responsible for either a focal compressive myelopathy, or it may result in a diffuse necrotic myelopathy. This has also been referred to as haematomyelia, ascending syndrome, haemorrhagic myelomelacia and ascending cord necrosis.

The exact pathogenesis of myelomalacia is poorly understood, but is now believed that vasospasm due to the release of vasoactive substances like histamine, serotonin and the kinins as well as venous occlusion and thrombosis result in progressive ischaemia. The onset is typical of that of a focal compressive myelopathy. Usually within 24 h of onset the cord lesion begins to progress. The pathological process may both ascend and descend along the cord, or either component may predominate. The neck is often held in extension, with the brows wrinkled and an anxious expression in the eyes, contributing to the impression that the dog is extremely apprehensive or in severe pain. In most cases death ensues within four days due primarily to respiratory failure.

DIAGNOSIS AND LOCALISATION OF LESION

The signs of intervertebral disc protrusions depend on the site and severity of spinal cord compression. Cervical disc protrusion is usually characterised by pain and muscle spasm with little or no signs of paralysis.

Both the thoraco-lumbar cord and the vertebral canal are smaller than in the cervical area. At least 80 % of the available space is occupied by the spinal cord. For this reason, the effects of disc protrusion or extrusion in the thoraco-lumbar area are more severe, with extensor rigidity or paralysis common signs. Urinary incontinence and faecal retention are also common

0038-2809/79/03/0201–0204

Looking at a cross section of the spinal cord, it can be seen that three major tracts are ventrally situated.

1. The pyramidal tracts are most centrally situated. They function in motor control.
2. The crossed vestibulospinal tracts inhibit tone of the extensors of the opposite side.
3. The direct vestibulospinal tracts increase extensor tone.

It is clear that if the pyramidal tracts are damaged, it will affect the co-ordinated movements of the legs in locomotion, leading to paresis or paraplegia. Damage to the crossed vestibulospinal tracts will remove the inhibitory control on the extensor or antigravity muscles. This will give rise to spasticity or spastic paresis or paralysis. When direct vestibulospinal tracts are also involved, the extensor tone is reduced with resultant flaccid paralysis.

Another very important tract to consider in evaluating the severity of cord damage, is the lateral spinothalamic tract. It contains pain and temperature fibres. Absence of deep pain sensation indicates a grave prognosis as it means that severe pressure was applied or radiated as far as the lateral spinal cord.

The lesions already mentioned are called upper motor neuron (UMN) lesions affecting the hing legs. Hypotonia usually occurs with lower motor neuron (LMN) disease, whereas UMN is usually characterised by hypertonia or spasticity. The functional integrity of the LMN is necessary for muscle cell contraction and to maintain muscle tone. It is also essential for the health of the muscle cells it innervates. When denervated, these cells degenerate, giving rise to neurogenic atrophy very rapidly. The UMN influences the activity of the LMN to produce voluntary motor function and to maintain muscle tone for support of the body against gravity.

In summary then, although the UMN includes both facilitatory and inhibitory functions on the activity of the LMN, when the UMN is diseased the result usually is a release of the LMN from inhibition and overactivity of the facilitatory mechanism. This release is seen as hypertonia or spasticity.

The lower motor neuron consists of the following components:–

1. Afferent neuron (sensory)
2. Interneuron/s in the grey matter of the cord.
3. Efferent neuron (motor) leaving the ventral horn.

The integrity of the lower motor neuron can be verified by testing various spinal reflexes, and as the cell bodies making up these LMNs to the limbs are located in very specific areasof the spinal cord, any lesion involving these specific LMNs can be fairly pinpointed in the cord.

It must be remembered however, that the spinal cord segments do not correspond to the vertebral bodies from L3–L7.

Abnormalities of the LMN will cause signs of muscle weakness – paresis or paralysis (flaccid) along with of hyporeflexia or areflexia, hypotonia or atonia, and rapid neurogenic atrophy.

Spinal reflexes are depressed or absent when there is loss of the motor component of the reflex arc, as muscle tone is dependent on the continual contraction of a small regulated number of muscle cells. When their motor innervation is lost, there can be no contraction to maintain muscle tone.

In intervertebral disc disease showing LMN signs, the lesion usually involves the nerve cell body in the ventral grey column owing to pressure and ischaemic necrosis. As is well known, these destroyed nerve cells are incapable of regeneration or replacement and for this reason LMN signs present a graver prognosis in the clinical case than an UMN lesion where nerve tracts are primarily involved.

Reflexes in localising a lesion:

Reflex	Peripheral nerve	Spinal cord segment	Level in vertebral canal
Patellar	Femoral	L4–5	Le–4
Flexor	Sciatic	L6–S1	L4–5
Perineal	Pudendal	S1–3	L5
Extensor-thrust	Femoral	L4–5	L3–4

Other tests performed

1. Muscle tone
2. Deep pain perception
3. Panniculus reflex:– the afferent components are the segmental spinal nerves and the efferent is via the lateral thoracic nerve (C8–T1) This reflex is only reliable from about L5 to the mid-thoracic region.

INTERPRETATION OF CLINICAL SIGNS

In 1953 and 1954 Tarlov and his co-workers published the results of an interesting study on the influence that the rate of onset of spinal cord compression had on recovery and the time available for successful decompression.

According to his findings, the following table serves as a useful guide.

Onset of total paralysis	Period of time after onset of complete paralysis within which decompression must be carried out for complete recovery
1. Sudden acute compression (within minutes)	2 h
2. Gradual compression	
(a) within 75 minutes	9 h
(b) within 20 hours	84 h
(c) within 48 hours	1 week

In a study by Finkquist in 1962, in which the neurologic examination i.e. pain perception, voluntary movements and muscle tone as well as the rate of onset of symptoms were correlated to the recovery rate with conservative treatment, the following valid conclusions were drawn:–

1. Where pain and voluntary movements are retained regardless of the rate of onset, recovery is usually complete.
2. Dogs which lose voluntary motor control, yet retain pain sensation, usually regain normal use of their legs.

3. Dogs which lose both pain sensation and voluntary movements, must have a return of these functions within 5 d from onset of complete paralysis to regain normal leg function. If they have not begun to walk within 10 d, there is little chance of complete recovery.
4. Loss of both voluntary movements and pain sensation for 2 weeks results in little chance of these patients ever regaining the use of their legs.
5. Dogs which progressively lose voluntary movements, muscle tone and pain sensation, i.e. flaccid paralysis in 12–24 h, either develop ascending paralysis or remain totally paralysed.
6 Dogs unable to use their legs fully after 9 months have little chance of every being normal.

RADIOGRAPHY

A thorough systematic clinical examination combined with the use of plain radiography can allow one accurately to pinpoint the offending disc in 90 % of the cases.

Radiography must be performed with the patient under general anesthesia. For best results very good quality X-rays taken with a grid are essential to be able to identify subtle changes such as fogging of the intervertebral foramina owing to extruded disc material, slight narrowing of collapsed disc spaces and early signs of calcification of the nucleus pulposus.

It should not be attempted to obtain a representative picture of the whole spine with a single radiograph. Two or three overlapping pictures of the thoraco-lumbar area should be taken in both the lateral and ventro-dorsal projections. If these radiographs are inconclusive as are the clinical signs in pinpointing the offending disc, myelography could be resorted to. A new contrast medium, Metrizamide will shortly be available. This drug is reputed to be safer than other preparations.

TREATMENT

From the above it is apparent that conservative treatment is not ideal for the patient with loss of both pain sensation and voluntary movement. Decompressive laminectomy would be the surgical treatment of choice. In fact this should be done in all cases where motor function is lost even though pain sensation is still present.

Surgical techniques

A Disc fenestration
B Dorsal laminectomy (a) narrow – Finkquist
(b) wide – modified
C Hemilaminectomy

As has been pointed out, time is the most important factor influencing the success rate in the surgical treatment of paralytic disc disease. Some of these cases should have surgery on an emergency basis. One should operate within 24 h of onset of paralysis with perhaps 48 h as the upper limit. Beyond this time limit the success rate can diminish drastically.

Equally important is an irreproachable atraumatic surgical technique in exposing and decompressing the cord. It serves little purpose turning a good clinical recovery risk into a permanent cripple, or worse, by traumatising the cord during surgery.

Wide dorsal laminectomy

This procedure is favoured because:–
(a) It facilitates total decompression of the cord.
(b) It allows for the removal of disc material from both sides of the cord, and even retraction of the cord sufficiently to examine the floor of the vertebral canal.
(c) If the laminectomy extends over only one or even two intervertebral spaces, the stability of the spinal column is not affected.

Technique

The patient is placed in sternal recumbency on the operating table with supports on either side of the body. Draping must be extensive to ensure complete surgical asepsis.

The initial skin incision is made just lateral to the midline and should extent about two vertebrae on either side of the target disc. The incision is continued through the subcutaneous fat to the thoracolumbar fascia, which is incised on either side of the spinous processes. The multifidus lumborum and longissimus muscles are bluntly dissected from the spinous processes with a sharp periosteal elevator. The muscle attachments to the articular processes are also bluntly freed. Some haemorrhage may be encountered here from dorsal branches of the intervertebral arteries. Suction and cautery are used to control haemorrhage.

Once the articular processes and the laminae are freed of muscle attachments and fully exposed, the spinous process on either side of the disc area to be decompressed is removed with bone cutters. Next, the caudal articular facets of the cranial vertebra are removed with stout rongeurs and the dorsal intervertebral space is carefully exposed. The nearest caudal spinous process is grasped with a towel clamp and by lifting on this clamp the intervertebral space is slightly opened, allowing a small rongeur to be introduced into the dorsal vault of the canal. By means of small bites of the rongeurs, the dorsal lamina is nibbled away cranially the full width of the cord. The cranial articular facets are not removed. The exposure is carried almost the entire length of the cranial vertebra. The rongeurs are then applied to the caudal vertebra in the same manner. Great care must be taken not to injure the cord.

Once the cord has been exposed for the required distance, extruded disc material can be removed from either side and even from below the cord with a small dental spatula or probe. Care must be exercised not to rupture the extensive venous sinuses. If the cord is severely compressed, a durotomy will further decompress the cord.

Before closing the incisions, a small pad of subcutaneous fat is placed on the exposed cord to protect it. orthopaedic wire is also strung like a 'clothes line' between the two spinous processes on either side of the laminectomy site. The thoracolumbar fascia is closed over this wire. The wire serves two function:– (a) it increases stability of the spine after surgery and (b) it conceals the deformity left by removal of the spinous processes. The fat and subcutaneous fascia are sutured and the skin is closed. A light bandage is applied for three days to prevent seroma formation. Skin sutures are left in for at least 14 d.; routine physiotherapy is commenced on the first post-operative day.

REFERENCES

1 Lahunta A 1977 Veterinary Neuroanatomy and Clinical Neurology. W B Saunders and Co., Philadelphia
2. Brown N O, Helpfrey M L, Prata R G 1977 Thoraco-lumbar disk disease in the dog: A retrospective analysis of 187 cases. Journal of the American Animal Hospital Association 13: 665–672
3 Finkquist B 1962 Studies on disk protrusion in the dog. Royal Veterinary College, Stockholm, Sweden
4. Finkquist B 1962 Thoraco-lumbar disk protrusion with severe cord compression in the dog. Acta Veterinaria Scandinavica 3, Parts I, II & III
5 Henry W B 1975 Dorsal decompressive laminectomy in the treatment of thoraco lumbar disc disease. Journal of the American Animal Hospital Association 11: 627–635
6 Knecht C D, Small E 1971 Diseases of the spinal Cord. In Canine Medicine. First Catcott (ed) American Veterinary Publications
7 Morgan J P, Suter P F, Hilliday T A 1972 Myelography with water soluble contrast medium radiographic interpretation of disc herniation in dogs. Act Radiologica (Suppl) 319: 217
8 Tarlov I M, Klinger H 1954 Spinal cord compression studies II. Time limits for recovery after acute compression in dogs. Archives of Neurology and Psychiatry 71: 271
9. Tarlov I M 1954 Spinal cord compression studies III. Time limits for recovery after gradual compression in dogs. Archives of Neurology and Psychiatry 71: 588
10 Trotter E J 1977 Canine intervertebral disc disease In: Kirk R W (Ed) Current Veterinary Therapy VI. Small Animal practice. W B Saunders and Co, Philadelphia.

BOOK REVIEW — BOEKRESENSIE

VETERINARY RADIOLOGICAL INTERPRETATION

S. W. DOUGLAS & H. D. WILLIAMSON
Heinemann Veterinary Books 1978. Price: R22.90

The re-appearance of this book (first published in 1970) in the bookshops is fortunate, since almost all veterinarians now possess an X-ray unit. Whilst they may understand the rudiments of radiology, the interpretation of radiological plates does present the non-specialist veterinarian with certain problems. This book is not the universal panacea but its attraction lies in the fact that it can help to overcome some of those problems. Furthermore, the inherent value of this book is that it presents in a single volume information that is distributed widely in other books and journal articles.

As a technical production, the reproduction of X-rays is more than adequate, although the choice of material could be improved upon in subsequent editions. This is a minor criticism in a book that has many good features. For instance, Section One is particularly valuable in that it relates technique and interpretation. Section Two, which discusses the radiology of the skeletal system, contains a very good section on age and bone and joint development. In this particular chapter, schematic diagrams as opposed to reproduced X-rays are used to outline these changes. Such diagrams are used in al the chapters of the book to relate radiological principles and the interpretation of normal and abnormal radiographs.

Whilst these examples represent some of the qualities of the book, the book is not without fault. One might be tempted to say that it does not go into enough detail. But from the viewpoint of the non-specialist veterinaria, one could view this feature as an asset, whereas the criticism is probably quite valid from the specialist's viewpoint. Secondly, the section on the mouth, teeth and salivary glands is quite meagre and if the authors are planning a new edition, this is one section in dire need of expansion. Thirdly, Section Three, which discusses radiology of the soft tissues, could have been improved upon if it had devoted more space on the practical aspects of contrast media e.g. type, quantity or dose and the interval times for exposures. In this regard it might be argued that this book serves as an aid to interpretation but the reviewer feels this information should have been included. Lastly, this book is primarily aimed at the small animal practitioner. Although some large animal radiology is included, this is not adequate in its present form.

In conclusion, this book is a worthwhile companion to the authors' first book "Principles of Veterinary Radiology" and should be a valuable asset to the practising veterinarian.

L B

CANINE CERVICAL INTERVERTEBRAL DISC DISEASE

G.E. FROST

ABSTRACT: Frost G E **Canine cervical intervertebral disc disease.** *Journal of the South African Veterinary Association* (1979) **50** No. 3 205–206 (En) Practitioner, Box 50258, Randburg 2125, Rep. of South Africa.
The incidence, diagnosis and surgical treatment of canine cervical disc lesions are described.

INTRODUCTION

Cervical intervertebral disc disease presents a clinical picture somewhat different from a thoraco-lumbar problem.

Most patients with thoraco-lumbar disc disease are presented for treatment only after they have developed some degree of motor dysfunction, whereas most patients with cervical disc disease are presented with pain symptoms and little or no evidence of paralysis.

Breed incidence

1. Dachshund
2. Pekingese
3. Miniature Poodle
4. Beagle
5. Miniature Schnauzer
6. Cocker Spaniel
7. Corgi
8. Alsatian

The breed incidence of cervical disc disease corresponds to that of thoraco-lumbar disc disease, with Dachshunds and Beagles being most frequently affected. The condition appears to be becoming more frequent in German Shepherds.

Site incidence

Although any of the discs can become affected, the C2–3 articulation has the highest incidence of disease, followed by C3–4.

SYMPTOMATOLOGY

Typically the patient is presented with signs of cervical pain accompanied by vocalisation, spasm of the cervical muscles and a disinclination for the animal to move its' head and neck.

Any manipulation of the head and neck is resented as it usually causes severe pain. These pain symptoms are primarily due to pressure on the spinal nerve roots. If the spinal cord is compressed, signs of neurologic deficit will appear, involving all four limbs. Pelvic limb paresis and ataxia usually are more severe than in the thoracic limbs, but occasionally the reverse may be true. In cases with more severe thoracic limb deficits, the extrusion has been largest on the midline, compressing more of the central portion of the spinal cord.[1]

DIAGNOSIS

The diagnosis is usually easily made from the history and the presenting symptoms. Diagnosis is confirmed in most cases by means of radiography. Calcification of the nucleus pulposus is usually seen with slight dorsal or dorsolateral elevation of the disc material. The intervertebral disc space may be reduced or it may be essentially normal with little or no herniated disc material visualised in the intervertebral foraminae.

TREATMENT

A Conservative

When clinical signs are very mild or transient, non surgical treatment regimens may be employed. e.g. Antispasmodics; Cortico-steroids; Analgesics; Ultra sound; Manipulation, etc.

Nevertheless, the clinical symptoms usually recur or may even become more severe unless the degenerated disc material is surgically removed.

B Surgical

Ventral fenestration is the treatment of choice for several reasons:–

1. Progression of herniation is prevented. Once the nucleus pulposus has completely herniated with disc material extruded against the cord, it becomes a more complicated clinical problem.
2. Relief of pain is prompt. Patients often recover from anaesthesia free of pain or become painfree within a few days. If some disc material has been extruded into the spinal canal, recovery will be delayed.
3. Surgery is simple and atraumatic. The procedure is quick, with minimal complications if the prescribed technique is followed. Prolonged treatment and recurrent attacks are eliminated. Very little post operative treatment is usually necessary.
4. Recovery is permanent. There should be no recurrence at the fenestrated discs since the nucleus pulposus is removed in each case and a stable fibrous union occurs.

   All discs from C2–3 to C6–7 should be fenestrated to avoid the possibility of unfenestrated discs causing future problems.

SURGICAL TECHNIQUE

The patient is placed in dorsal recumbency in an operating rack or between supports with the head slightly lower than the shoulders and with a pad under the neck. This allows good access as far caudally as C6–7.

0038-2809/79/03/0205–0206

A midventral skin incision is made from the larynx to the manubrium sternum. The incision is carried through the subcutis. By means of blunt dissection, the *M. Sternocephalicus* is separated from the *M. Sternothyrohyoideus* on the side of the operating surgeon. The trachea, oesophagus and carotid sheath with its associated blood vessels and nerves are retracted to the side away from the surgeon. The retraction must be done gently to avoid trauma to the vessels and nerves.

By "cleaning up" some areolar tissue, the longus colli muscle bundles covering the ventral surface of the vertebrae in a herring bone pattern are now exposed.

Careful palpation will allow the identification of the ventral prominence of the caudal aspect of each vertebral body at the point where the longus colli muscle fibres insert. The intervertebral disc is located immediately caudal to this prominence.

A small stab incision is made just caudal to the ventral prominence, transecting some longus colli muscle fibres. These muscle bundles are moved caudally by blunt dissection, exposing the anulus fibrosus. Lateral bundles are retracted laterally with thumb forceps to facilitate removal of a ventral wedge of anulus which is cut out with a no. 15 or no. 11 Bard Parker scalpel blade. The nucleus pulposus is carefully curetted out, care being taken not to penetrate too deep and enter the spinal cord or to force some calcified disc material into the canal. Haemorrhage is usually minimal and is easily controlled.

On completion of the procedure, the muscles are not sutured. The subcutis and skin are closed routinely.

POST OPERATIVE CARE

The animal is often painfree immediately after surgery. If discomfort is present, corticosteroids and muscle relaxants can be administered. Antibiotics are not administered routinely. The patient is usually hospitalised for two or three days and is then kept reasonably confined at home for two weeks to allow fibrous union to occur in the intervertebral disc spaces.

Occasionally an animal may show signs of discomfort for longer periods of time if some disc material continues to irritate a nerve root. In such cases one should locate the disc and re-operate to clear it out.

BIBLIOGRAPHY

1. De Lahunta A 1977 Veterinary Neuro-anatomy and Clinical Neurology W B Saunders Co., Philadelphia pp 169–220
2. Trotter E J 1977 Canine intervertebral disc disease In Kirk R W (ed) Current Veterinary Therapy VI Small Animal Practice. W B Saunders Co., Philadelphia. pp 841–848

CASE REPORT **GEVALVERSLAG**

CANINE PNEUMOCYSTIS PNEUMONIA

R.M. McCULLY*, JUNE LLOYD**, DALE KUYS** and D.J. SCHNEIDER**

ABSTRACT: McCully, R.M.; Lloyd, June; Kuys, Dale; Schneider D.J. **Canine pneumocystis pneumonia.** *Journal of the South African Veterinary Association* (1979) **50** No. 3 207–213 (En) Animal Unit, UCT Medical School, Observaroty 7925, Rep. of South Africa.

This is a case report of pneumocystosis of an eight month old Dachshund from the Cape Province. Clinically it was an afebrile disease with signs limited primarily to the lower respiratory tract. The report consists of a short history, the histopathologic findings, evidence of the electron microscopic confirmation of the diagnosis and a brief discussion. It is believed to represent the first case of canine pneumocystosis in the Republic of South Africa.

INTRODUCTION

Pneumocystis pneumonia is a sometimes fatal disease of humans caused by *Pneumocystis carinii,* an organism of uncertain taxonomy which has been found in rodents, lagomorphs and a number of domestic animals including the dog. Cases of pneumonia caused by *P. carinii* have been reported in man and a goat[2] in South Africa but this is, to our knowledge, the first reported canine case in South Africa. Although the organism has been generally accepted as a protozoan until recently, there is now considerable support for the contention that there is a closer relationship of pneumocystis to fungi of the class Ascomycetes, subclass Hemiascomycetidae[1]. The organism has not been cultured *in vitro.*

CASE HISTORY

In October 1976 an 8 months old Dachshund suffering from severe respiratory distress was treated with antibiotics by a veterinarian in Stellenbosch. Three days later, having shown no improvement, the dog was brought to the Regional Veterinary Laboratory at Stellenbosch for diagnostic tests. The dog was afebrile and severe respiratory distress was the only sign. Blood was collected and except for an elevated haematocrit (48 %) and increased percentage of white cells (3 %), other tests results were within normal limits. It was advised that another veterinary clinician's opinion should be obtained. The following day the dog was given an antibiotic by a practitioner in Cape Town but it died in the owner's car on the way home and was taken to the Regional Veterinary Laboratory for a *postmortem* examination.

AUTOPSY

The most significant finding was the diffusely affected lungs. They had a grey-white marbled appearance and an increased consistency and did not collapse when the chest cavity was opened. There were no fully consolidated areas. Bacteriological cultures of the lung were negative.

*Animal Unit, UCT Medical School, Observatory 7925
**Regional Veterinary Laboratory, Stellenbosch.
(Submitted for publication on 27/6/79)

Methods

Representative pieces of lung were fixed in 10 % formalin after which sections were cut from paraffin embedded tissue using routine procedures. Sections of lung were initially stained with haemotoxylon eosin (HE) but following their examination, additional sections were stained with periodic acid schiff (PAS) and Grocott-Gomori methanamine silver (GMS).

Microscopic findings

Lung

There was a remarkable interstitial pneumonia with a marked thickening of the alveolar septa caused by the presence of large mononuclear cells with rather vesicular nuclei. There was some evidence of early fibroplasia in the alveolar septa. The alveolar spaces were narrowed by the impingement of the thickened alveolar septa and further compromised by the presence of more mononuclear cells, some of which had confluent cytoplasm and formed multinucleated giant cells. Many of the alveoli also contained a weakly eosinophilic substance of foamy appearance (Fig. 1). Because of the thickened alveolar walls, the accumulations of cells in alveolar spaces and the amorphous alveolar material, the lung had a very consolidated appearance with obviously little opportunity for the normal exchange of gases (Fig. 2). There was hyperplasia of alveolar lining cells and considerable metaplasia towards a cuboidal appearance as seen in the foetal lung (foetalisation). A few polymorphonuclear leukocytes were scattered about and in some instances concentrated in alveolar ducts. Large macrophages with foamy cytoplasm filled some of the alveolar spaces.

Closer analysis of the HE sections showed that the less consolidated in appearance, the more of the foamy or honeycomb appearing substance there was in the alveoli. When the GMS stained sections were studied, organisms consistent with *P. carinii* were easily demonstrated (Fig. 3). The appeared as oval to round argyrophilic bodies approximately 8 μ in diameter (Fig. 4). Although many appeared to be empty, others contained one or two argyrophilic bodies about 1–2 μm in diameter. Many of the large macrophages contained ar-

0038-2809/79/03/0207–0213

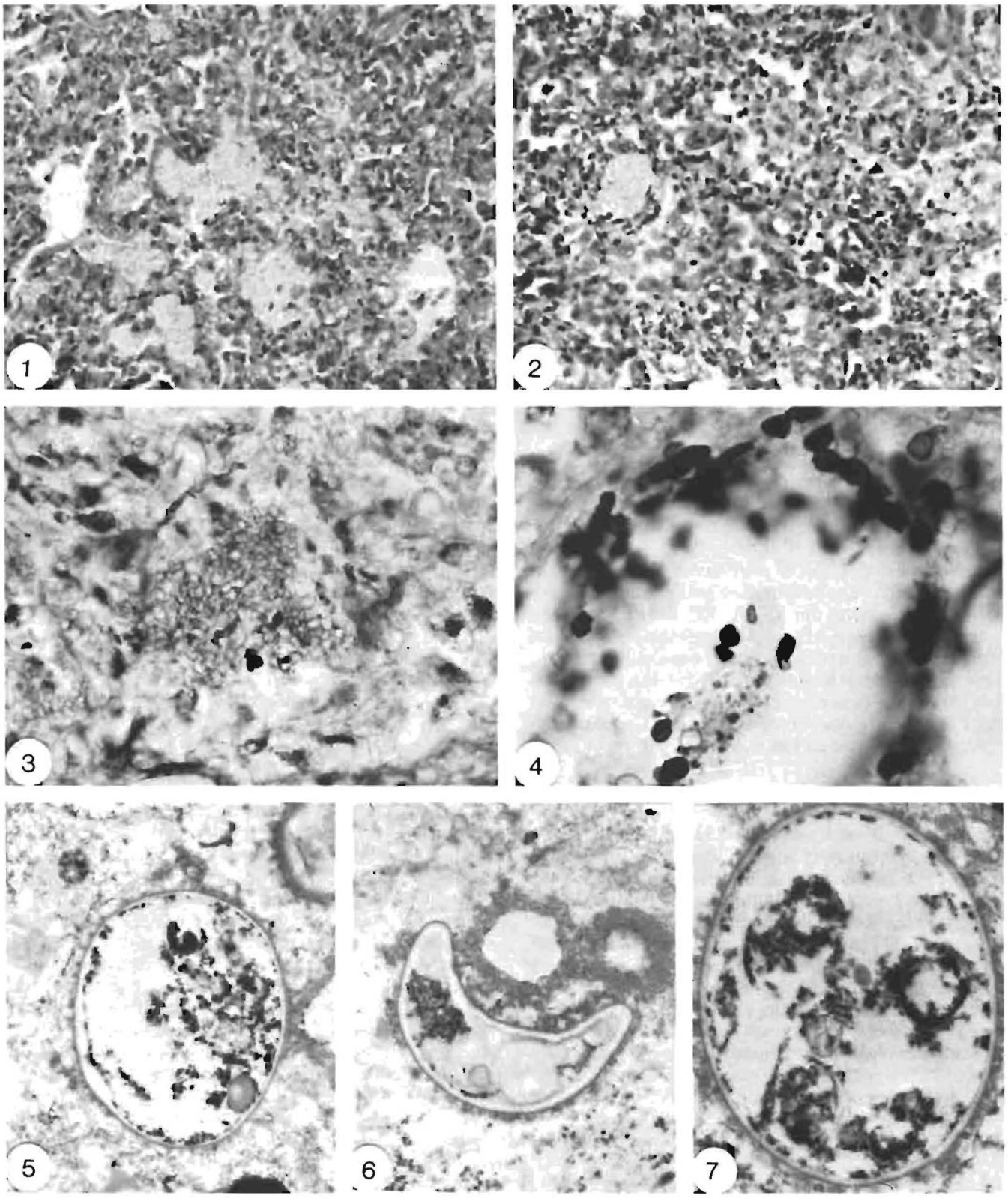


Fig. 1. The alveoli frequently contained weakly eosinophilic foamy appearing material. Lung, HE x 125.

Fig. 2. Other alveoli were filled with macrophages and other leukocytes. Lung HE x 125.

Fig. 3 Cystic stages of *Pneumocystit carinii* are well demonstrated with silver, but the bulk of the organisms shown here are in the trophic stage. Lung, GMS x560.

Fig 4. A higher magnification of cysts of *P. carinii.* Lung, GMS x1400.

Fig. 5. An early cystic form of *P. carinii* was demonstrated. EM x10800.

Fig. 6. A crescent-shaped remnant of a ruptured cyst is shown. EM x10800.

Fig. 7 A cystic form of *P. carinii* containing 4 intracystic bodies is shown. EM x10800.

gyrophilic debris in their cytoplasm. The PAS reaction demonstrated an even greater number of *P. carinii* organisms forming a honeycomb-like pattern in alveoli.

Confirmation by electron microscopy

Thin sections from formalin fixed tissues were cut using routine techniques for EM study. Examination of these sections revealed various forms of *P. carinii,* the best demonstrable being early cysts (Fig. 5), the crescent-shaped remains of ruptured cysts (Fig. 6) and poorly preserved cystic form of the organism containing four intracystic bodies (Fig. 7). The trophic stage of the organism was the most numerous seen but the EM pictures were disappointing because of poor preservation.

DISCUSSION

Todate, the report by Farrow, Watson & Hartley[1] is the definitive paper on this disease in the dog and will be drawn on heavily in the next few paragraphs for relevant information. These authors reported a series of 6 cases, all in male, miniature dachshunds between 9 and 12 months of age. They were all presented with dyspnoea although afebrile and alert. Examination of radiographs showed a diffuse increase in radio-density of the lungs and cardiac enlargement.

The most consistent haematological findings were a leukocytosis with neutrophilia and a monocytosis, an increased haematocrit and a polycythemia in 5 of the 6 dogs.

Significant gross findings at post-mortem examination were essentially the same for all the dogs and were considered by the authors to be characteristic for the disease. The lungs, which did not collapse when the chest was opened, had a firm, rubbery consistency, were pale and contained patches of yellow discolouration. The cut surface was similarly discoloured, appeared to be consolidated but portions of lung floated in the fixative.

Histologically, the following changes were regarded as being characteristic: foamy material in alveoli and bronchioles, distended alveoli, alveolar macrophages and neutrophils, alveolar epithelialization, rupture of alveolar septa and parenchymal collapse, bronchiolar inflammatory exudate and interstitial pneumonitis. Of these the most outstanding feature was the foamy material in alveoli and the terminal bronchioles. PAS stained the foamy material in alveoli as a pale to bright pink honeycomb. These plentiful organisms were not well demonstrated with the Grocott-Gomori staining method; nevertheless there were numerous argyrophilic cyst-like bodies about 8 μm in diameter scattered amongst them.

With electron microscopy, the alveoli were found to be crowded with trophic stages of the organism. More circular forms representing precystic stages as well as cystic stages containing up to 8 intracystic bodies were seen. Ruptured cysts appeared as crescent shaped remains. The authors were surprised at the absence of degenerative changes in the alveolar walls.

The present case is reminiscent of many of the features of the cases so well described by them. It was clinically very similar, also being a Dachshund though not a miniature and almost the same age. The foamy contents of alveoli that stain better with PAS than with GMS appear to contain mainly the trophic stage of the organism while GMS demonstrates the early, the well-developed and the ruptured systs best.

Farrow *et al.*[1] point out that although the causative organism has been found in a long list of laboratory and domestic animals, there are few reports of naturally occurring pneumocystosis in animals. They further pointed out that most clinical infections in man occur in premature and debilitated children which are probably immunologically incompetent as well as in adults similarly deficient and that a relationship between pneumocystosis and immune deficiency states has been demonstrated in laboratory animals. They further suggest that pneumocystosis may be more likely to occur in dogs suffering from congenital or acquired immune deficiency states.

They suggest that pneumocystis pneumonia be considered when a young dog suffers severe dyspnoea and weight loss but is alert and afebrile. Further, it appears to be a fatal disease in such dogs if untreated. They found a good clinical response to treatment with pentamidine isothionate. They warn of severe necrosis and ulceration at the intra-muscular injection site of this drug and, in view of a probable underlying immune deficiency, suggest a guarded prognosis for the long term.

Pneumocystosis of man is increasing in significance and prevalence in patients which are debilitated by a number of disease states or iatrogenic procedures[3]. In view of the evidence quoted by some authors[1] that the disease is a zoonosis, the owner of an affected dog may choose not to have the dog treated, especially if either he or his family might fall into either of these groups or are otherwise immunologically compromised.

REFERENCES

1. Farrow B R H, Watson A D J, Harley W J 1972 Pneumocystis pneumonia in die dog. Journal Comparative Pathology 82: 447
2. McConnell E E, Basson P A, Pienaar J G 1971 Pneumocystosis in the goat. Onderstepoort Journal Veterinary Research 38: 117
3. Rippon J W 1974 Medical Mycology, the Pathogenic Fungi and the Pathogenic Actinomycetes p 462 W.B. Saunders Co. Philadelphia

CASE REPORT GEVALVERSLAG

TOXOPLASMOSIS IN A DOG

J VAN HEERDEN & I B J VAN RENSBURG

ABSTRACT: Van Heerden, J.; Van Rensburg, I B J **Toxoplasmosis in a dog.** *Journal South African Veterinary Association* (1979) **50**, No. 3 211–214 (En) Faculty of Veterinary Science, University of Pretoria, P O Box 12580, Onderstepoort 0110.

The clinical, clinical pathological and pathological findings of a dog showing progressive paralysis due to toxoplasmosis are described. Necropsy revealed polymyositis and a meningoencephalitis as well as a meningomyeloradiculitis. The differential diagnoses of the different forms of toxoplasmosis are briefly discussed.

INTRODUCTION

Acquired as well as congenital forms of toxoplasmosis have been reported in the dog[7]. A large percentage of dogs in many countries show a positive serological titre to *Toxoplasma gondii*[3]. Most dogs develop asymptomatic infections and are latent carriers of the parasite. This latent state can be changed to overt disease by a lowering of the host's resistance[10]. Whenever clinical symptoms develop there is a wide variation in the way the disease manifests itself in the dog. In addition, immaturity and a concurrent distemper infection might also increase the susceptibility of dogs to toxoplasmosis[10]. The epidemiology, life cycle, pathogenesis of toxoplasmosis and comparative pathology have been reviewed by Turner[9 10]. In contrast to some other animal species dogs do not represent an important source for toxoplasmosis transmission as it is believed that they do not shed oocysts and are seldom consumed by man or other animals[3].

This report deals with the clinical symptoms, clinical pathological observations and post mortem examination of a dog suffering from toxoplasmosis.

History, clinical and clinical pathological findings

A 6-month old Labrador-male was referred to the Department of Medicine, Faculty of Veterinary Science, Onderstepoort with a history of progressive hind limb weakness. Approximately 2 months previously the owners had noticed that the pup walked with a marked sway. According to them, the animal's hind limbs were tucked in under its body with adduction of the hocks and abduction of the hind feet. On occasion it was unable to raise its hindquarters. The condition gradually became worse although high doses of cortisone during this period apparently caused some relief of the symptoms.

On physical examination the dog was found to be bright and alert and had no fever. Tremors were present in the muscles of the head and face. The animal assumed a sitting position and was unable to raise its hindquarters. The muscles of the hind legs were slightly atrophic. Barking was high-pitched.

Neurological examination showed the cranial nerve reflexes to be present. There was a flaccid paralysis of the hind legs. The placing reaction was present in the left front leg, weakly present right front and absent in both hindlegs. The extensor postural thrust, wheelbarrow, hemistand and hopping reactions could be elicited in the front legs only. Weak flexor and patellar reflexes were present in the hind legs whilst the extensor reflex was absent. The panniculus reflex was present up to the first lumbar vertebra. At this stage the dog was able to control urination and defecation. However, the dog became progressively more paralytic during the ensuing 2 weeks until eventually (i) only the head and head and neck could be lifted; (ii) total aphonia set in; (iii) there was urinary incontinence and; (iv) stasis of the bowels with constipation.

The leg muscles were now atrophic and no reflexes were present in any of the legs. Some perception of pain was still present as the dog lifted its head following pinching of a toe. Its appetite remained good.

The results of haematological examination, blood chemistry tests and examination of cerebrospinal fluid (CSF) are listed in Table 1. Radiographic examination of the entire vertebral column did not reveal any abnormality as did fluoroscopic examination of the vertebral canal following the injection of a radio-opaque dye.

The dog died following the fluoroscopic procedure and a full post mortem examination was done.

Table 1: CLINICAL PATHOLOGICAL EXAMINATION RESULTS

	Patient	Normal range
Blood		
Hb g/l	134	120–180
RCC 1 x 10^{12}/l	6,12	5,5–8,5
Ht	0,47	0,37–0,55
MCV f	75	60–80
WCC 1 x 10^9/l	9	6–17
Neutrophiles	0,62	0,60–0,77
Lymphocytes	0,31	0,12–0,30
Monocytes	0,05	0,03–0,10
Eosinophiles	0,02	0,02–0,10
SAP U/l (25°C)	57	50–122
SGOT U/l (25°C)	43	15–30
SGPT U/l (25°C)	75	8–25
CPK U/l (26°C)	347	0–70
CSF		
App	Clear	Clear
SG	1,007	1,003–1,012
Prot. g/l	5	0,11–0,55
Gluc m mol/l	2,9	3,4–6,4
WCC 1 x 10^9/l	98	to 30

0038-2809/79/03/0211–0214

POST MORTEM EXAMINATION

Mascroscopic findings

The most conspicious macroscopic lesion was a severe, wide spread myopathy involving especially the longissimus dorsi, muscles of the hind limbs, the neck and diaphragm. The affected muscles had a mottled appearance due to area of suspected Zenker's hyaline degeneration and numerous ecchymoses.

The myocardium was not affected. The lungs were congested and oedematous with focal haemorrhages. Congestion, oedema and petechiae were observed in the retropharyngeal lymph nodes. The mesenteric lymph nodes were also oedematous. Congestion and white pulp hyperplasia of the spleen were present. No macroscopic lesions were noticed in the brain and spinal cord.

Microscopic findings:

Brain

Haematoxylin and eosin stained sections from the cerebral cortex, central white matter of the cerebellum and cerebrum, thalamus, cerebral peduncles and medulla were examined. An outspoken meningoencephalitis or encephalitis was present in every section examined. The most prominent lesions were perivascular cuffing of mainly plasma cells and lymphocytes, encephalomalacia with necrosis of neurones, some gitter cell proliferation and swollen axis cylinders. *Toxoplasma* pseudocysts and ruptured cysts disseminating tachyzoites into the brain tissues were regularly encountered. There was a diffuse increase in the number of round cells in the meninges and in some areas of the brain several eosinophils and macrophages were present. Hyaline degeneration of the walls of some of the blood vessels were observed as well as leucostasis in some blood vessels.

Spinal cord

Outspoken meningomyelitis and focal meningoradiculomyelitis were observed in the spinal cord. The lesions were more prominent in the myelinated portion of the cord and was of the same nature andintensity as in the brain. The grey matter was also affected. Lesions of the same degree and severity were present from the certical, to the sacral areas of the spinal cord. Radiculitis was mild and focal affecting only a few of the nerve roots. These roots exhibited degenerative changes with a round cell infiltration but no *Toxoplasma* organisms were found in the affected nerve roots although they were easily found in the spinal cord.

Skeletal muscle

There was extensive involvement of the skeletal muscles with lesions varying from a Zenker's hyaline degeneration and necrosis to calcification of necrotic foci and marked fibrous replacement of muscular tissues. In the necrotic areas macrophage infiltration was evident, proliferating sarcolemma nuclei were located centrally in some fibres and atrophic fibres were a common finding. In other foci plasma cell infiltration dominated the scene, usually in the company of small numbers of eosinophils. Intramuscular pseudocysts of *Toxoplasma* were present in many of the affected areas – some of these had ruptured with dissemination of organisms while other containing very small organisms with pyknotic nuclei appeared to be degenerating.

Histopathological examination of the liver, kidney and sciatic nerve did not reveal any significant lesions. Unfortunately the myocardium and lymphoid tissues were not examined microscopically.

Table 2: SUMMARY OF SOME OF THE MICROSCOPIC FINDINGS

Lesion	Miningitis	Encephalitis	Myelitis	Radiculitis
Severity of lesion	++	+++	+++	+

DISCUSSION

Both the acute and chronic forms of toxoplasmosis are difficult diagnostic entities. Ehrensperger, Suter[2] recognised 3 different forms of toxoplasmosis in young dogs, namely: (i) a generalized form in pups 7–12 months old, (ii) a central nervous system for in pups 4 months and older, (iii) a radiculoneuritis form in pups younger than 3 months of age. The generalized form of toxoplasmosis is characterized by symptoms such as intermittent fever, tonsillitis, dyspnoea, diarrhoea, vomition and a mucopurulent yellowish nasal and ocular discharge. Sharma and co-workers describe cases showing small distemper-like papules on the belly[6]. The central nervous sytem form is characterised by symptoms of disturbance of the central nervous system and by histopathological changes in the central nervous system and perhaps the spinal chord. The radiculoneuritis form is characterised by a progressive paresis and paralysis with histopathological changes most frequently in the nerve roots. An encephalitis, myelitis, myositis and myocarditis were also described.

The cases of radiculoneuritis as described by Ehrensperger and Suter[2] were all in pups 1–3 months of age and were regarded by them as a typical form in young dogs.

In the presented case the symptoms of progressive paresis and paralysis were more typical of the radiculo-

LEGENDS TO FIGURES

Fig 1. Encephalitis showing perivascular cuffing, round cell infiltration and swollen axis cylinders (arrows) x 200.

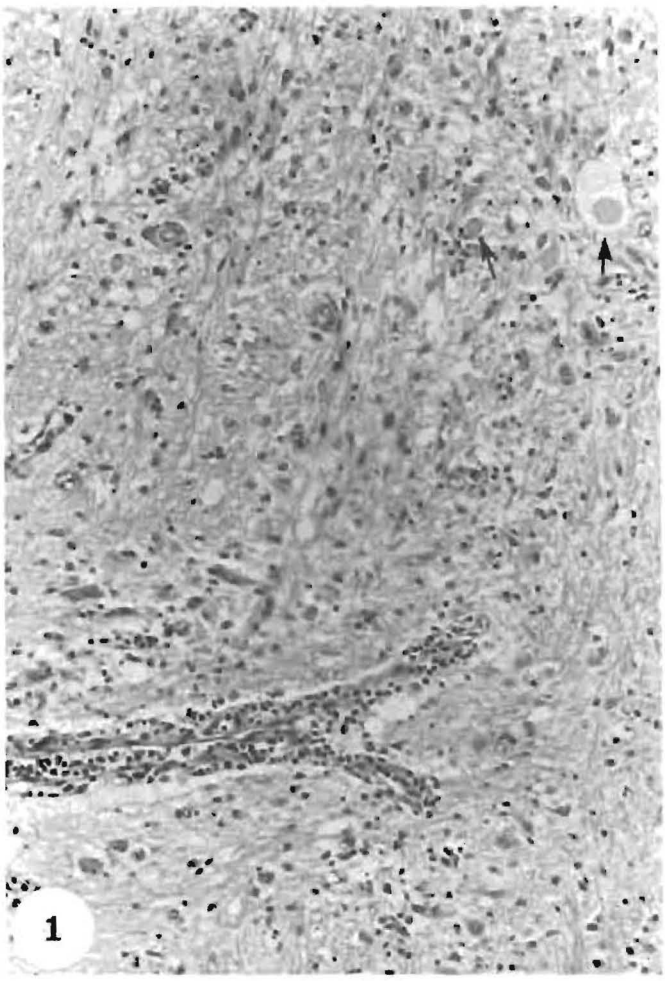
Fig 2. *Toxoplasma* "pseudocyst" in brain (arrow) x 800.

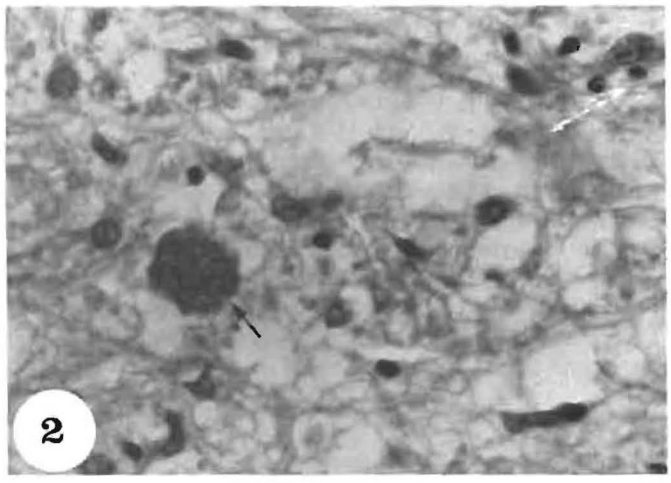
Fig 3. Spinal nerve root showing radiculitis with degeneration and mild round cell infiltration x 200.

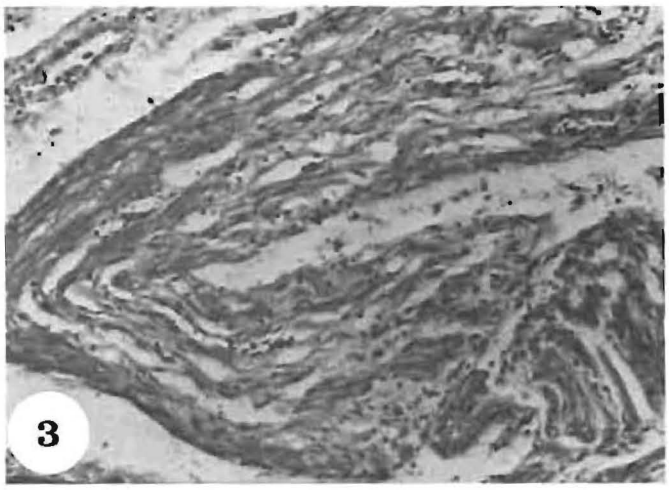
Fig 4. Skeletal muscle with macrophage and polymorph infiltration into the muscle fibres x 1200.

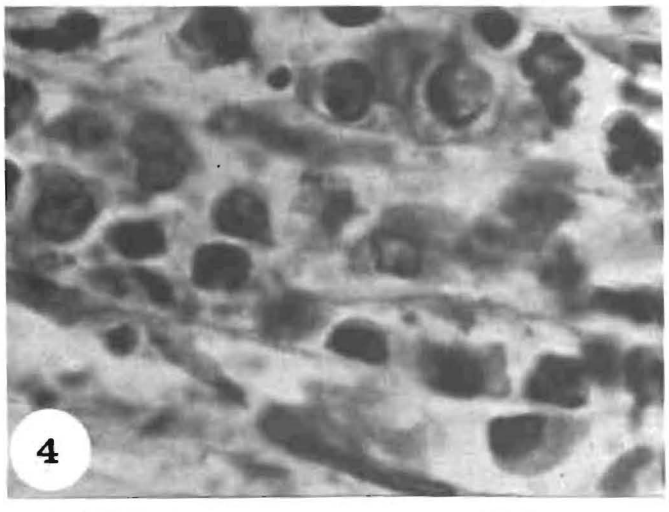
Fig 5. Chronic focal myositis with calcification x 80.

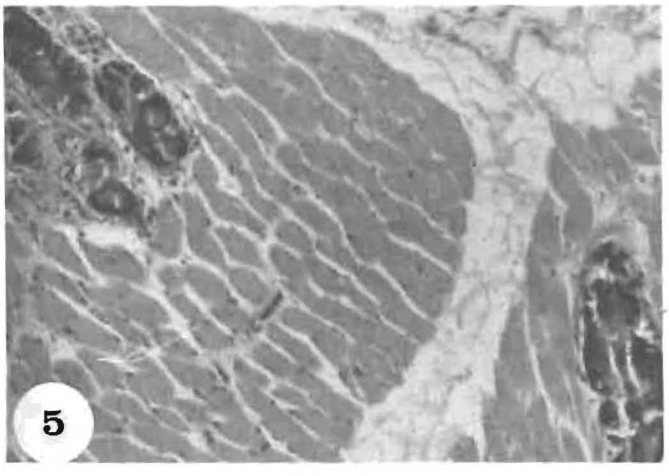
Fig 6. Myositis with degenerated muscle fibre (bottom) round cell infiltration, proliferation of sarcolemmal nuclei (white arrow), early fibrosis, collapse of fibres and a *Toxoplasma* pseydocyst (black arrow) x 800.

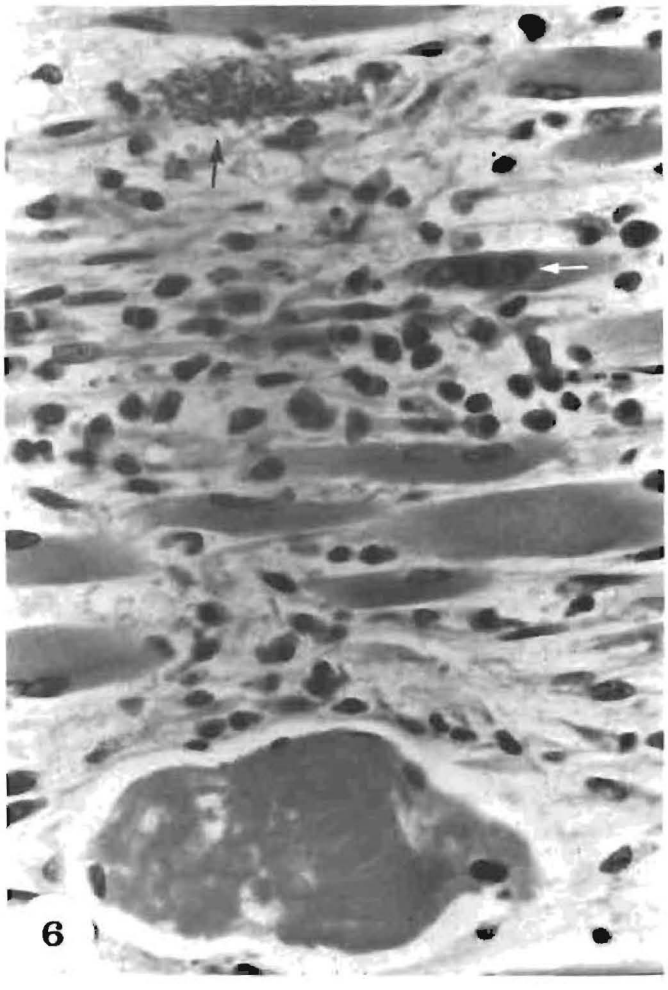

1


2


3


4


5


6

neuritis form although the dog was slightly older. The lesions in the nerve roots were less extensive and less severe than lesions elsewhere. (Table 2).

Increased CPK and SGOT levels were indicative of a muscular lesion. An elevated total protein content as well as a raised white cell count in the cerebrospinal fluid suggested an inflammatory or degenerative process in the central nervous system. The cerebro spinal fluid in toxoplasmosis of the central nervous system is usually abnormal[1].

Evaluation of serum antibody titre against *Toxoplasma gondii* as well as examination of cerebrospinal fluid-sediment might have facilitated ante-mortem diagnosis[1].

REFERENCES

1 Averill D R, De Lahunta A 1971 Toxoplasmosis of the canine nervous system: Clinicopathologic findings in four cases. Journal of the American Veterinary Medical Association 159: 1134–1141

2 Ehrensperger F, Suter M 1977 Radiculitis toxoplasmica beim Hund Kleintier-praxis 22: 59–61

3 Martin M, Letarte R 1977 Prevalence of toxoplasmosis in Quebec farm dogs. Veterinary Record 101: 79–80

4 Palmer A C, Barker J 1974 Myasthenia in the dog. Veterinary Record 95: 452–454

5 Perchman G E 1976 Rhipicentor infestation in the dog: a case report. Rhodesian Veterinary Journal 7: 15–16

6 Sharma S P, Guatum O P, Kharole M U 1973 Studies on some aspects of pathogenesis, chemotherapy and serology of experimental toxoplasmosis in dogs. Indian Veterinary Journal 50: 626–633

7 Smit J D 1961 Toxoplasmosis in dogs in South Africa: Seven case reports, Journal of the South African Veterinary Medical Association 47: 227–231

8 Trayser C V, Marshall A E 1974 A mild form of polyradiculoneuritis in a dog. Journal of the American Veterinary Medical Association 164: 150–151

9 Turner G V S 1976 toxoplasmosis as a public health hazard. Journal of the South African Veterinary Association 47: 227–231

10 Turner G V S 1978 Some aspects of the pathogenesis and comparative pathology of toxoplasmosis. Journal of the South African Veterinary Association 49: 3–8

GEVALVERSLAG **CASE REPORT**

HEPATOZOÖNOSE IN 'N KAT

S. VAN AMSTEL

ABSTRACT: Van Amstel S. **Feline hepatozoonosis.** *Journal of the South African Veterinary Association* (1979) **50** No. 3, XXX–XXX (Afr. En) Dept. Medicine, Fac. Vet. Science, University of Pretoria, Box 0110 Onderstepoort, Rep. of South Africa.

The presence of the gametes of the protozoon parasite *Hepatazoon* in the blood of a domestic cat is recorded for the first time in South Africa. Clinical symptoms which may have been associated with the infection are described. The animal recovered after treatment with primaquine and oxytetracycline.

GESKIEDENIS

Die gevalverslag het betrekking op 'n agtien maande oue, manlike huiskat wie se eienaar in Warmbad woonagtig is. Op 1978.08.04 het die eienaar opgemerk dat die kat lusteloos was en nie wou eet nie. Die kat is na 'n privaat veearts geneem wie 'n diagnose van stomatitis gemaak het. Die kat is behandel met "Ampiclox"* en multivitamienstroop per os. Die mondletsels is lokaal behandel met 'n jodium-gliserien preparaat. Die kat se dieët het op hierdie stadium bestaan uit kommersiële ingemaakte katkos. Ses dae later het die praktisyn besluit dat die kat nie op behandeling reageer nie en is die kat na die Fakulteit Veeartsenykunde, Onderstepoort verwys.

KLINIESE BEVINDINGS EN BEHANDELING

Met opname het die kat lusteloos voorgekom met 'n dowwe, glanslose haarkleed. Daar was ligte kliniese tekens van dehidrasie teenwoordig. Die rektale temperatuur was 41 °C maar dis as misleidend beskou aangesien die kat ondersoek is kort na 'n motorrit van Warmbad na Pretoria. Die hartspoed en respirasie was binne normale perke. Ondersoek van die mondholte het slegs 'n ligte gingivitis getoon waar die tandvleis in kontak met die tande was. Die kiestande in hierdie areas was bedek met 'n matige hoeveelheid tandsteen. Geen onmiddellike behandeling is noodsaaklik geag nie.

Een dag na opname was die kat se algemene kondisie onveranderd. Die rektale temperatuur was egter nou 39,2 °C. Die kat is 'n ligte algemene narkose toegedien dmv 'n binneaarse kortwerkende barbituraat†. Al die tandsteen is verwyder en die mondslymvlies ontsmet met 'n mondspoelmiddel. Steriele deppers vir bakteriologie is van die areas van gingivitis geneem vir kultuur en antibiogram. Die kat het goed van die narkose herstel.

Gedurende die daaropvolgende 4 d het die gingivitis goeie tekens van herstel getoon maar die kat se algemene kondisie het staties gebly; die dier was lusteloos en het geen eetlus getoon nie. Daarbenewens het die kat se temperatuur op Dag 5 weer begin styg. (Tabel 1). Die kat se stygende temperatuurreaksie, lusteloosheid en anoreksie het geen verband met die verbeterde

Tabel 1: TEMPERATUUR, HABITUS, EETLUS EN BEHANDELING VAN KAT MET *HEPATOZOON*-BESMETTING

Dag	Temperatuur	Habitus	Eetlus	Behandeling
1	41 °C	Swak	Geen	Geen
2	39,2	Swak	Geen	Mond
3	38,8	Swak	Geen	Mond
4	39,0	Swak	Geen	Mond
5	39,8	Swak	Geen	Mond
6	40,6	Swak	Geen	Oksitet
7	39,4	Swak	Geen	Oksitet
8	40,2	Swak	+	Primaquine + Oksitet
9	40,8	Beter	+++	Oksitet
10	39,8	Beter	++	Oksitet
11	39,6	Beter	++	Oksitet
12	39,2	Goed	++++	Oksitet
13	38,8	Goed	++	Oksitet
14	38,4	Goed	++++	Oksitet

mondtoestand gehou nie. Verdere kliniese ondersoeke kon egter geen lig werp op die oorsaak van hierdie kliniese simptome nie. Bloed is geneem vir hematologiese ondersoek en die uitslag is vervat in Tabel 2. *Hepatozoon*-parasiete is in die neutrofiele in die bloedsmeer opgemerk. Aangesien geen ander oorsaak vir die kliniese tekens gevind kon word nie, is aanvaar dat die parasiet moontlik daarvoor verantwoordelik kon wees. In die lig hiervan is besluit om die kat as volg te behandel: Oksitetrasiklien* per os teen 'n dosis van 50 mg per kg b.i.d. vir 7 d asook primaquine (8-amino-quinoline), 2 mg per os totale dosis vir slegs een behandeling (Tabel 1). Die oksitetrasiklienbehandeling is begin op Dag 6 na opname maar die primaquine is eers toege-

*Beechams
†"Intraval" (May Baker)

*"Liquamycin" (Pfizer)

0038-2809/79/03/0215–0216

Tabel 2: KLINIES-PATHOLOGIESE BEVINDINGS OP DAG 5

Hemoglobien	(g/l)	137
Rooiseltelling	(10^{12}/l)	6,47
Hematokrit		0,51 ↑
Witseltelling	(10^{9}/l)	13,9
Neutrofiele	(volwasse)	0,89 ↑
Limfosiete		0,08 ↓
Monosiete		0,01
Eosinofiele		0,01

dien op Dag 8. Vanaf Dag 9 het die kat se habitus en eetlus merkbaar begin verbeter maar die temperatuur op hierdie stadium was nog 40,8 °C (Tabel 1). Vanaf Dag 10 het die temperatuur begin daal en was normaal vanaf Dag 12. Hematologie en smere is herhaal op Dag 14 met negatiewe resultate; geen *Hepatozoon* parasiete kon in die bloedsmeer demonstreer word nie. Aangesien die kat toe klinies normaal voorgekom het is daar besluit om hom te ontslaan. Ses maande na behandeling het die kat volgens sy eienaar steeds heeltemal normaal voorgekom.

MORFOLOGIE VAN DIE PARASIET

Morfologies het die gamete ooreengestem met die van *Hepatozoon canis*. Die gamete in die leukosiete het voorgekom as ovaalvormige liggame, omtrent 8–12 by 3–6 mikron in grootte, met 'n kompakte kern wat dikwels eksentries geleë was. Die sitoplasma het geen kleuring getoon met "Cam's Quick-stain" oplossing nie terwyl die kerne lig tot matig pers-rooi gekleur het. Met Giemsakleuring kom die sitoplasma ligblou voor en bevat dikwels menige klein donkerblou tot pers granules terwyl die kern donker rooi-pers kleur.

BESPREKING

Hepatozoon spp. kom wydverspreid in verskeie soogdiere, reptiele en voëls voor en is veral algemeen onder knaagdiere. Die parasiet is nie voorheen in Suid-Afrika in die huiskat beskryf nie, maar wel in wilde felidae insluitende die Luiperd en Cheetah[1]. Sover vasgestel kon word is *Hepatozoon* nog slegs een keer voorheen in die huiskat beskryf; in 1908 het Patton gevalle in huiskatte gevind in Madras[2]. Die parasiete wat voorkom in die kat, jakkals, hond en die wolf en wat respektiewelik bekend staan onder die naam van *H. felis, H. rotunda, H. canis* en *H. chattoni,* is feitlik nie van mekaar onderskeibaar nie en is volgens Levine[2] heelwaarskynlik dieselfde spesie.

In die genus *Hepatozoon* vind schizogonie in die viscera van 'n gewerwelde gasheer plaas terwyl die gamete in die leukosiete (meestal neutrofiele) of rooibloedselle afhangende van die spesie gebind word[2]. Bevrugting en sporogonie vind plaas in 'n ongewerwelde gasheer (bosluis, luis, muskiet, tsetsevlieg of ander bloedsuiende insekte). Die gewerwelde gasheer raak besmet deur die eet van die ongewerwelde gasheer. In die geval van *H. canis* (wat dus die kat sou insluit) is die ongewerwelde gasheer die hondehokbosluis *Rhipicephalus sanguineus*[2].

Die voorkoms van *Hepatozoon* is welbekend in die hond waar dit dikwels gepaard gaan met ander besmettings soos *Erlichia canis* en *Babesia canis. Hepatozoon*-infeksie in die hond word dikwels as nie-patogeen beskou alhoewel verskeie patologiese letsels wel met die toestand geassosieer word[1,2]. Kliniese simptome in die hond wat met die parasiet geassosieer word, sluit die volgende in: fluktuerende temperatuurreaksies, progressiewe vermaering, anemie en tumor splenis[2]. In hierdie geval was die uitgesproke simptome ook 'n fluktuerende temperatuur, swak habitus en totale verlies van eetlus. Die milt was nie palpeerbaar nie en daar was geen anemie nie. Daar was wel ligte neutrofilie maar laasgenoemde word gewoonlik assosieer met stresstoestande in die kat.

Sover vasgestel kan word is nog geen gekontroleerde behandelingseksperimente uitgevoer met *H. canis* nie. In hierdie geval is dit egter baie duidelik dat die kat kort na aanvang van behandeling tekens van klinies herstel getoon het. Verder kon geen *Hepatozoon*-parasiete vanaf die 6-de dag na toediening van die primaquine demonstreer word nie en vir ses maanda na behandeling het die kat steeds heeltemal gesond voorgekom.

VERWYSINGS

1. McCully R M, Basson P A, Bigalke R D, de Vos V, Young E 1975 Observations on naturally acquired hepatozoonosis of wild carnivores and dogs in the Republic of South Africa. Onderstepoort Journal of Veterinary Research 42: 117–134
2. Levine N D Protozoan Parasites of Domestic Animals and of Man. (2ed) Burgess Publ. Co., Minneapolis.

TECHNICAL NOTE **TEGNIESE AANTEKENING**

DETERMINATION OF PENICILLIN RESIDUES IN MILK – A COMPARISON OF TWO METHODS

K.W. KATZ and R.C. COOK

ABSTRACT: Katz K.W.; Cook R.C. **Determination of penicillin residues in milk – a comparison of two methods.** *Journal of the South African Veterinary Association* (1979) **50** No. 3 217–211 (En) City Health Department, Box 1477, Johannesburg 2000, Rep. of South Africa.

Two tests for penicillin residues in milk using the test organisms *Sarcina lutea* and *Bacillus stearothermophilus var. calidolactis* respectively are presented. The test methods are described, the results compared and the advantages of the latter test demonstrated.

INTRODUCTION

Information gained from the South African Veterinary Association's Public Health Group in 1976 indicated that a lack of uniformity exists from laboratory to laboratory in testing milk for thermoresistant inhibitory substances, thus making correlation of results on a national basis almost impossible.

The purpose of this paper is to attempt to create greater uniformity of testing methods and techniques and to compare the results of two methods employing two different penicillin sensitive organisms, *Sarcina lutea* and *Bacillus stearothermophilus var. calidolactis.*

METHODS

Method I

For this method the *S. lutea (Micrococcus luteus)* agar paper disc diffusion was used. This has been the standard method employed by the Johannesburg Municipal Milk Laboratory[1] since 1958 and has a sensitivity to Na-penicillin G of 0,007–0,01 international units per m*l* milk.

Method II

The Thermocult rapid agar diffusion method was developed by Orion Diagnostics in co-operation with the Food Hygiene Department of Veterinary Medicine of the University of Helsinki, Finland. This method involves the use of prepared agar plates containing an enriched medium with a pH indicator and a standard volume of stabilised *B. stearothermophilus var. validolactis* spores. The sensitivity of this method is claimed to correspond to 0,0025 international units of Na-penicillin G per m*l* milk[2].

Preparation of *Sarcina lutea* plates

1. A culture of *S. lutea* No. 9841 was obtained from the Medical School of the University of the Witwatersrand. The culture was maintained alternately on Pen Assay Seed (PAS) agar and nutrient agar slopes and sub-cultured at least 4 times weekly to ensure a rapidly growing culture.
2. A suspension of *S. lutea* was prepared by washing the growth from a 24 h incubated PAS agar slope culture with 4 m*l* of sterile physiological saline.
3. 0,4 m*l* of this suspension was added to 100 m*l* of melted PAS agar maintained in a fluid state at a temperature of 48 °C. The suspension was well mixed avoiding any air bubbles.
4. With a 10 m*l* pipette having a large bore tip, 5 m*l* of the seeded agar was transferred rapidly to prewarmed (48 °C) petri dishes with a diameter of 100 mm, distributed evenly and allowed to solidify on a level surface.

Provision of Thermocult* plates

For this method commercially produced plastic Thermocult plates were used. These plates can be stored for considerable periods under the correct conditions and are immediatey available for use.

Thermocult reference discs with a diameter of 12,7 mm containing 0,001 iu Na penicillin G per disc corresponding to 0,01 iu penicillin per m*l* were also supplied. These are used to control the Thermocult working procedure and sensitivity so as to be able to compare inhibitory zones obtained to international units of penicillin in milk. Thermocult reference discs will produce a zone of inhibition of 18–19 mm in diameter. Thermocult plates stored as recommended by the manufacturer will remain active and accurate for 4 months and the reference discs for a period of 2 months[2].

Preparation of Milk Sample

Fresh antibiotic free undiluted milk was treated to a temperature of 80 °C for 5 minutes to destroy any naturally occurring inhibitory substances. The necessity of this procedure to destroy naturally occurring inhibitory substances is debatable[3] but is important from a public health point in the detection of thermoresistant inhibitory substances (TRIS).

Preparation of the Penicillin Dilutions

300 mg ampoules of dried laboratory standard Na-benzyl penicillin containing 1 500 iu per mg or a total of

*Combined Medical Specialities, Johannesburg.

0038-2809/79/03/0217-0211

450 000 iu were acquired. One ampoule was re-constituted with 4,5 m*l* sterile distilled water to give a stock dilution of 100 000 iu/m*l* = A.

Serial dilutions were made as follows in antibiotic free milk:

B: 1 cc of A + 9 cc = 10 000 iu/m*l*
C: 1 cc of B + 9 cc = 1 000 iu/m*l*
D: 1 cc of C + 9 cc = 100 iu/m*l*
E: 1 cc of D + 9 cc = 10 iu/m*l*
F: 1 cc of E + 9 cc = 1 iu/m*l*
G: 0,7 cc of F + 0,3 cc = 0,7 iu/m*l*
H: 0,5 cc of F + 0,5 cc = 0,5 iu/m*l*
I: 0,3 cc of F + 0,7 cc = 0,3 iu/m*l*
J: 0,3 cc of F + 2,7 cc = 0,1 iu/m*l*
K: 0,7 cc of J + 0,3 cc = 0,07 iu/m*l*
L: 0,5 cc of J + 0,5 cc = 0,05 iu/m*l*
M: 0,3 cc of J + 0,7 cc = 0,03 iu/m*l*
N: 0,3 cc of J + 2,7 cc = 0,01 iu/m*l*
O: 0,7 cc of N + 0,3 cc = 0,007 iu/m*l*
P: 0,5 cc of N + 0,5 cc = 0,005 iu/m*l*
Q: 0,3 cc of N + 0,7 cc = 0,003 iu/m*l*
R: 0,3 cc of N + 2,7 cc = 0,001 iu/m*l*
S: 0,7 cc of R + 0,3 cc = 0,0007 iu/m*l*

Preparation of Assay Discs

The assay disc preparation was carried out in duplicate.

Whatman AA (Antibiotic Assay) discs of 13 mm diameter with an absorption capacity of 0,1 m*l* were used. Pairs of discs corresponding to penicillin dilutions in the milk sample from 1 iu/m*l* to 0,007 iu/m*l* were placed on 25 mm x 25 mm squares marked out on a glass plate. By means of a 1 m*l* pipette 0,1 m*l* of milk to be tested was pipetted onto corresponding filter discs commencing with the lowest dilution to avoid the possibility of transferring penicillin from a higher to a lower dilution. The pipette was thoroughly rinsed out with water between samples.

An additional two assay discs were saturated with 0,1 m*l* of milk containing 1 iu/m*l* of penicillin to which 0,05 m*l* of penicillinase (sufficient to inactivate 1,875 Mega units of Na-penicillin) was added. The absence of any inhibition in the penicillinase treated sample was accepted as positive indication that the inhibitory effect obtained in the other discs was due to penicillin.

Two Thermocult reference discs each saturated with 0,1 m*l* of distilled water were used to compare their inhibition zones with those of filter discs impregnated with milk containing 0,01 iu pencillin.

The transfer of the saturated filter discs to their respective petri dishes was carried out within 10–15 minutes of the milk pipetting process to avoid dessication.

By means of forceps the pairs of saturated filter discs were lifted individually from the glass plate and transferred first to the *S. lutea* inoculated plates and then onto the Thermocult plates. Transfer of the discs was from the lowest to the highest concentration of penicillin. The forceps were rinsed and dried between each disc. Filter discs inoculated with milk containing 0,05 iu/m*l* and more penicillin were placed on individual petri dishes while those with lower concentrations several on one plate. However, care was taken that each disc was at all times more then 20 mm from the edge of the petri dish and 20 mm from each other to avoid overlapping of the inhibitory zones.

One Thermocult reference disc and one assay disc saturated with milk containing penicillinase were placed respectively on a *S. lutea* plate and a Thermocult plate.

The filter discs were carefully pressed onto the agar surface with the forceps to ensure close contact.

Incubation

I. *S. lutea* inoculated plates

The surface contact of the assay disc to the agar medium was further enhanced by allowing the petri dishes to remain on the laboratory bench for 5 minutes after which the petri dishes were placed in an inverted horizontal position in an incubator for 18 h at a temperature of 37 °C,

The zones of inhibition were very clear and the diameters were measured with a calliper.

II. Thermocult plates

The same procedure was adopted for these plates with the exception that the petri dishes were incubated for 3 h at a temperature of 65 °C. Stacking of petri dishes one on top of the other was avoided to ensure uniform temperature distribution and to maintain the horizontal level of the petri dish.

The incubation period could be decreased by 40–50 minutes by the pre-incubation of the plates at 65 °C for 1 h before placing the assay disc on the medium.

Inhibition characterised by a purple zone around the disc was measured by callipers. The remainder of the plate was clearly yellow. In order to observe the inhibition zone distinctly the plate should be held at various angles to the light. Each test was repeated five times.

RESULTS

The results are summarised in Table 1.

DISCUSSION

1. From the results obtained, the Thermocult method proved very accurate and reproducible. The ability to detect a specified residual level of penicillin and to quantify this level accurately to international units of penicillin is possible. The reproducibility of the *S. lutea* method did not achieve that of the Thermocult method. From our previous experience and that of other workers, *S. lutea* has a tendency to lose its sensitivity to penicillin. This has been ascribed to a mutation characteristic of the organism.
2. The Thermocult method is extremely rapid and simple to perform as the seeded plates are ready prepared. Any suspicion of antibiotic adulteration of a milk consignment can be confirmed within 3 hours or less. A minimum amount of equipment and training is necessary to perform the test and it must thus have its uses even in a commercial dairy to screen milk for TRIS. The *Sarcina* method, on the other hand, entails prior preparation, is considerably more difficult to perform, requires more expertise and has a minimum incubation period of 18–24 h.
3. The limit of detectability with the Thermocult method has been claimed to be 0,0025 iu of penicillin G/m*l* milk[3]; this was conclusively proved by the investigation. The test results and our previous ex-

Table 1: COMPARISON OF SIZE OF INHIBITORY ZONES OBTAINED IN THE *S. LUTEA* AND THERMOCULT TESTS

	Penicillin dilution in milk iu/m*l*	Sarcina method diameter of zone of inhibition in mm							Thermocult method diameter of zone of inhibition in mm						
		Test 1	Test 2	Test 3	Test 4	Test 5	Mean	Standard deviation	Test 1	Test 2	Test 3	Test 4	Test 5	Mean	Standard deviation
F	1.0	40	37	42	41	41	40,2	1,92	33	31	34	34	31	32,6	1,51
G	0,7	38	35	40	38	38	37,8	1,78	31	29	32	32	30	30,8	1,17
H	0,5	36	31	39	37	36	35,8	2,94	30	28	30	31	30	29,8	1,09
I	0,3	34	30	38	32	34	33,6	2,84	29	28	30	29	29	29,0	0,70
J	0,1	28	25	31	27	30	28,2	2,38	28	26	28	27	26	27,0	1,00
K	0,07	25	24	29	26	26	26,0	1,87	27	25	25	26	25	25,6	0,08
L	0,05	23	22	27	24	23	23,8	1,83	24	24	23	25	25	24,2	0,83
M	0,03	20	20	22	21	22	21,0	1,00	23	23	22	23	23	22,8	0,11
N	0,01	16	14	20	17	16	16,6	2,19	21	20	21	20	20	20,4	0,13
O	0,007	15	–	15	14	–	–	–	20	19	20	18	17	18,8	1,30
P	0,005	–	–	14	–	–	–	–	18	18	18	17	15	17,2	1,30
Q	0,003	–	–	14	–	–	–	–	17	17	17	15	15	16,2	1,09
R	0,001	–	–	–	–	–	–	–	14	14	14	14	–	–	–
S	0,0007	–	–	–	–	–	–	–	14	–	14	–	–	–	–
Thermocult Reference Disc		15	15	16	14	15	15	0,07	20	19	20	19	19	19,4	0,50

perience confirms that *S. lutea* is generally more variable and not sensitive to less than 0,01 iu or at the extreme 0,0007 iu penicillin G per m*l* milk.

CONCLUSION

The penicillin concentration of 0,003 iu/m*l* milk is considered harmful to persons sensitised to penicillin[5].

Controlling penicillin contaminated farm milk at the 0,01 iu/m*l* level is generally sufficient. This involves little risk to the consumer due to dilution of the farm milk by bulking of supplies in road tankers and at the manufacturing dairy.

However, when dealing with the final consumer product of pasteurised, UHT, powdered milk and other processed milk products the situation must be considered at the level of 0,003 iu/m*l* penicillin[4]. Here the necessity of a more accurate and reproducible standard test such as the thermocult method is evident.

ACKNOWLEDGEMENT

Thanks are due to the Medical Officer of Health, Johannesburg, for permission to publish this paper and to Dr M M Greathead for revising the manuscript.

REFERENCES

1. Meara P J 1959 A survey of penicillin contamination of market milk supplies. South African Medical Journal 33: 821–822
2 Galesloot T H, Hassing F 1962 A rapid and sensitive paper disc method for demonstration of antibiotics and chemo-therapeutic and Dairy Journal 16: 89–95
3 Pekhanen T J, Grunwald-Stenius V 1974 The Thermocult rapid metthod for demonstration of antibiotics and chemo-therapeutic agents in milk. Suomen Eläinlääkärilehti, 80: 205–211
4. Hakkarainen H, Haakka J 1973 Trial control of antibiotic contents of producer milks in 1973 Laboratory of Valio, Helsinki, Finland.
5. FIL – IDF: Annual Bulletin 1963, Part V Chemical Residues in Milk p 65, Square Vergote, Brussels.

TECHNICAL NOTE TEGNIESE AANTEKENING

THE PRESERVATION OF *CLOSTRIDIUM CHAUVOEI* IN LIQUID NITROGEN

CHERYL M.E. McCRINDLE

ABSTRACT: McCrindle Cheryl M.E. **The preservation of *C. chauvoei* in liquid nitrogen.** *Journal of the South African Veterinary Association* (1979) **50** No. 3 221–222 (En) Box 12472, Onderstepoort 0110, Rep. of South Africa. The preservation of vaccine challenge material in liquid nitrogen is described.

INTRODUCTION

When *Clostridium chauvoei* is serially propagated in liquid culture it sometimes loses its pathogenicity for guinea pigs. This complicates the standardization of challenge doses for vaccine potency tests. Stalheim[2] used liquid nitrogen to preserve *Leptospira interrogans* and it was felt that it might also be used for the preservation of *C. chauvoei.*

MATERIALS

Von Hibler's culture medium, modified after van Drimmelen[3], as prepared at the Onderstepoort Institute of Veterinary Research.

400 g minced beef; 250 g minced liver; 1 *l* of distilled water; 1,74 g KH_2PO_4; 2,82 g Na_2HPO_4 $12H_2O$; 10 g peptone.

Boil the meat and liver for one hour. Add salts and peptone. Adjust to pH 7,6–7,8. Filter. Autoclave for 60 minutes.

Add 10 g glucose to every litre of filtrate. Mince bovine brain coarsely and place in large test tubes to approximately one-third of their length. Add the above-described broth to approximately two-thirds the length of the test tube. Stopper the tubes and sterilise by tyndallisation. *C. chauvoei* strain 64 GP is a strain maintained at the Onderstepoort Institute of Veterinary Research and periodically passaged in guinea pigs to maintain its virulence.

METHOD

A freeze dried culture of *C. chauvoei* 64 GP was activated by growing overnight in von Hibler's medium. It was then checked for purity by streaking out on blood agar plates which were incubated both aerobically and anaerobically. At the same time 1 m*l* of the culture was inoculated into each tube of von Hibler's medium and incubated at 37 °C for 18 hours.

This actively growing vegetative culture was filtered through cheese cloth and placed in one m*l* quantities in freeze drying tubes. The tubes were heat sealed and individually wrapped in aluminium foil (this is a safety precaution as they occasionally burst when removed from the liquid nitrogen). They were then placed in the gas phase of a liquid nitrogen container.

As may be seen below (Table 1), the addition of 10 % glycerol or the adjustment of the pH to 7 prior to freezing decreased the virulence of the *C. chauvoei* for guinea pigs.

Table 1: THE INFLUENCE OF VARIOUS ADDITIVES ON THE VIRULENCE OF *C. CHAUVOEI*

Additive	MLD before freezing	MLD after freezing
None (medium only)	10^{-5}	10^{-5}
pH adjusted to 7	10^{-4}	10^{-4}
90 ml culture + 10 % glycerol	10^{-4}	10^{-2}

For challenge purposes three tubes were removed from the liquid nitrogen, thawed for 30 minutes, opened and mixed. As indicated in Table 2 below, the best results were obtained when distilled water was used for the serial dilution of challenge material.

Table 2: INFLUENCE OF VARIOUS FLUIDS USED FOR THE SERIAL DILUTION OF CHALLENGE

Diluent used	Remarks
Saline (0,85 % NaCl)	MLD 10^{-5} in guinea pigs
Phosphate buffered saline pH 7,2	Precipitated when $CaCl_2$ added. No deaths.
Distilled water	MLD 10^{-5}
Tryptone water	MLD could not be calculated

A test tube shaker was used to agitate the mixture for 30 s between each dilution in order to prevent clumping. Tryptone water increased the clumping and this might have been the cause of the random results obtained when this diluent was used.

After dilution and just before injection, one third by volume of 2.5 % w/v $CaCl_2$ was mixed with the challenge material[1]. Guinea pigs given 2 m*l* of this mixture intramuscularly were then observed for 48 h and the MLD calculated as the lowest dilution at which all the guinea pigs died.

0038-2809/79/03/0221–0222

RESULTS AND CONCLUSION

Prior to freezing the MLD was calculated at 10^{-5}. Subsequent testing was carried out as indicated below in Table 3.

Table 3: VIRULENCE OF CHALLENGE MATERIAL STORED IN LIQUID NITROGEN

Freezing time	% survival of guinea pigs						
	10^{-1}*	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	MLD
24 hours	0	0	0	0	0	50	10^{-5}
30 days	0	0	0	0	010	20	10^{-4}
12 months	0	0	0	0	010	20	10^{-4}

*Reciprocal of dilution of challenge material.

These results suggest that storage in liquid nitrogen is a satisfactory method of preserving *C. chauvoei* challenge material.

REFERENCES

1 Personeus G R, Cooper M S, Martini F V 1957 Studies on Blackleg Bacterins. *Cornell Veterinarian* 47: 361–371

2. Stalheim O H V 1971 Viable avirulent *Leptospira interrogans* serotype *pomona* vaccine: preservation in liquid nitrogen. *Applied Microbiology* 22: 726–727

3. Van Drimmelen G C 1960 Grepe uit die Afrikaanse Handleiding by die Bakteriologie. 2de Uitgawe. Wallachs, Pretoria

DIE STEDELIKE VEEARTS – 'N VERLEENTHEID OF GELEENTHEID?

Die afgelope tyd is heelwat menings gelug oor die vestiging van veeartse in stedelike gebiede. Hierdie menings het van oningeligtes sowel as ingeligtes gekom.

Ons het dit goed gedink om die saak 'n slag van die ander kant af te bekyk. Die meeste menings wat ons tot dusver gehoor het, het krities teenoor die situasie gestaan en die stedelike veaarts het swygsaam gebly.

In vandag se wêreld van dialoog sou 'n paar ander gedagtes dus nie onvanpas wees nie. Ons wil die saak aan die hand van die volgende stellings bespreek.

Stelling 1 – Die trek na die stad is aan die gang

Die veearts is nie die enigste beroep wat tekorte op die platteland ondervind nie. Dokters, spesialiste, ingenieurs, predikante e.a. toon duidelike konsentrasies in stede. Party skole loop leeg en sommige plattelandse skole staar sluiting in die gesig. Selfs die swartman konsentreer graag om stede. Die ergste van alles; baie boere het al die pad stad toe gevolg.

Die ontvolking van die platteland is 'n voldonge feit totdat die tendens sy optimum peil bereik het. Syfers wat bereken word dui dat teen die eeuwending 'n ontstellend lae aantal plattelandse inwoners sal oorbly.

In die lig van bg. is die trek van die veearts na die stad net 'n logiese gevolg. Waarom sou die meerderheid van die professie nou teen die stroom in platteland toe trek? As die stad ook sy werksgeleenthede bied met al die voordele van die stad, waarom na areas trek waar nadele dikwels die voordele oortref? Ons het geen beswaar teen diegene wat graag op die platteland WIL werk nie en elke veearts kan sy eie heil uitwerk waar dit hom die beste pas. Die platteland het immers ook op sekere gebiede sy voordele.

Die beskuldiging wat egter sommer uit die lug na 'n groot deel van die professie geslinger word, dat hulle net troeteldiere wil dokter, is 'n totaal skewe beeld. Die veearts wat in die stad gesetel is, bedien in elk geval 'n baie wyer spektrum as slegs troeteldiere. Ons sal eerder wou sê dat die stadsveearts wat *slegs* honde en katte dokter dalk nie in die meerderheid is nie. Daar is baie stadspraktyke wat 'n groot bydrae tot die platteland lewer.

Daarom wil 'n mens ook beswaar maak teen 'n onlangse opname wat aandui dat slegs 57 veeartse die platteland bedien. Hierdie syfers is uit verband geruk as die stadsveearts wat ook die platteland bedien nie ingereken word nie.

Daar is ook 'n neiging om intensiewe boerdery nader aan stede te konsentreer. Nabyheid van die mark en korter vervoerafstande speel hier 'n rol. Hierdie boerderye word weer 'n ideale bedieningsveld vir die stadsveearts. Dieselfde geld vir baie perde, wat ook deur stadsveaartse bedien word.

Ander voorbeelde van stedlike veeartse is volop, soos bv. die staatsveearts. Die stede het 'n redelik groot konsentrasie staatsveaartse wat in goed toegeruste kantore en fasiliteite 'n doeltreffende diens lewer. Baie van hierdie stedelike gesetelde staatsveeartse lewer nogtans hulle belangrike bydrae tot die platteland. Nog heelwat ander stadsveeartse ontsnap gereeld die beskuldigings, bv. abattoirhoofde, dieretuinveeartse, navorsers, akademici, veeartse in industrieë, perde-praktisyns, selfs veeartse wat betrokke is in wildbewaring, proefdiereenheid-veeartse, e.a.

Die beskuldigings word dus gewoonlik op die gewone privaat-praktiserende veearts in die stad gerig, terwyl ons ander kollegas in die stad rustig met hulle werk kan voortgaan. Dit is duidelik dat die platteland nie noodwendig die gebied is waar die veearts sy grootste bydrae kan maak nie. Die veld is wyd en die aanvraag in die stede is hoog.

Die feit is egter dat die stad se voordele sy nadele vir die meeste mense oortref. Dit is dus wat ons betref onbillik om van die veeartsprofessie te verwag om teen die algemene tendens in groot getalle na die platteland terug te trek.

Stelling 2 – Die veeartse werk vir mense alhoewel hy met diere werk

In 'n gesprek met 'n mediese dokter, het ons op 'n keer gepraat oor 'n kat wat ons in die nag moes behandel. Sy opmerking was feitlik 'n uitroep van verbasing, nl. dat hy tog vir 'n kat een uur in die nag sou opstaan! Ons kon dadelik antwoord dat ons dit ook kwalik sou kon doen, maar dat 'n mens dit graag vir die *eienaar* van die kat sou wou doen. Hierdie belangrike benadering word so dikwels vergeet, veral deur buitestaanders.

Die volgende analoë situasie kan die idee dalk verduidelik. Die dokter staan in die nag op vir sy pasient (mens) wie se behoeftes bevredig word en waarvoor die dokter vergoed word. Die veearts staan in die nag op vir sy kliënt (die mens): die kliënt se behoeftes word bevredig deurdat sy dier behandel word en hy vergoed die veearts daarvoor.

Die loodgieter staan tog nie in die nag op vir die waterpyp wat gebars het nie maar iemand moes tog die loodgieter geroep het. Hy bevredig dus weer sy klant se behoefte en die klant vergoed hom daarvoor. Net soos die loodgieter nie vir waterpype werk nie, werk die veearts ook nie vir diere nie.

Die veearts se waarde en werk sal beoordeel moet word aan die behoefte wat die *mens* aan sy dienste heg. Die veearts mag dalk 'n onsuksesvolle behandeling toepas maar as die kliënt tevrede is, sal hy terugkom. Die veearts kan 'n uitstekende behandeling toepas maar as die kliënt met die veearts ontevrede is, sal hy na iemand anders gaan.

Geen dier kan aan klop vir hulp, besluit of die behandeling goed genoeg was en of hy sal terugkom of die veearts vergoed nie. Daar moet egter dadelik bygevoeg word dat veeartse wel ook 'n groot verantwoordelikheid teenoor die dier het. In die eerste plek vir die lewe self, maar ook as eiendom van iemand anders wat aan jou toevertrou is.

Daarom sal daar tog in die meeste gevalle 'n korrelasie wees tussen die hantering en behandeling van die dier self en die tevredenheid van die kliënt. Of anders gesê: die veearts sal nie met foefies hom slegs kan instel om die kliënt te beindruk sonder om die dier ook werklik in ag te neem nie.

As die meerderheid mense nou stad toe trek, trek die vee tog nie ook stede toe nie. Alles waar, maar die groot getalle vee gaan nog nie die veearts uitroep vir dienste nie, maar die eienaars van die vee wel. Deurdat die kliëntetal verminder, word minder veeartse benodig. Die behoefte aan veeartse word wêreldwyd meesal bepaal deur die getalle van die veestapel te bereken. As ons stelling egter waar is dat veeartse VIR mense en MET diere werk, lyk die berekening van veestapelgetalle nie meer 'n akkurate aanduiding van veearts-tekorte te wees nie. Daar kan miljoene stuks vee wees, maar as daar nie 'n eienaar is wat die veearts se dienste aanvra nie, sal die privaat veearts saam met die vee krepeer. Die alternatief is gratis of gesubsideerde dienste wat weer 'n las op die Staat lê.

Miskien lê die probleem dan eintlik by die veeboer wat die lewe van die veearts op die platteland moet verseker en nie by die veegetalle nie. Lê hier dalk 'n opvoedingstaak op iemand se weg?

Dit bly dus asof stelling 2 waar is, dat die veearts se trek van of na die platteland grootliks beinvloed sal word deur die aanvraag van vee-eienaars i.p.v. veegetalle.

Stelling 3 – Die verspreiding van veeartse is 'n natuurlike seleksie en geen kunsmatige pogings gaan daaraan verskil maak nie.

Die feit bly staan dat as 'n veearts afgestudeer het, hyself en hy alleen sy professionele rigting bepaal. Die diversiteit van ons beroep het geen bekendstelling nodig nie. Van hierdie verskeidenheid van rigtings word 'n groot getal in en om stede bedryf. Om met sentimentele argumente die veearts te probeer oortuig om wel platteland toe te trek, sal nie opgaan nie.

'n Mens kry soms die idee dat die plattelandse veearts as die groot patriotte aangeprys word, dat hulle alleen sou help om na die voedsel van die volk om te sien, dat hulle die pioniers is en dat die plattelandse veearts die ideale diens is wat die professie behoort te lewer. Van hierdie gedagtes mag waar wees, maar ons sal 'n baie groot fout maak as ons die werk van stedelik gesetelde veeartse as enigsins minderwaardig sou beskou.

Soos reeds aangedui, lewer stadsveeartse ook hul bydrae tot die veebedryf. Tweedens is 'n hele aantal sg. plattelandse veeartse so betrokke by die landbou dat hulle self deeltyds of voltyds boer. Vir die professie is hulle dus deels of heeltemal verlore. Derdens is genoem dat slegs 15 % boere 85 % landbouprodukte lewer. Wat die syfer vir veeboere is, weet ons nie, maar as die veearts platteland toe moet trek vir die 85 % onekonomiese boere, is dit, om die minste te sê, te veel verwag.

As die veearts dan besluit om wel sy heil in die stad te gaan soek, al doen hy ook wat in die stad, moet daar tog seker goeie redes voor wees. Die stadsveearts lewer ook sy gemeenskapsdiens, bevorder ook die wetenskap en bou aan die goeie beeld van die professie.

Die plattelandse veearts lewer nie noodwendig 'n groter bydrae tot die gemeenskap of professie as die veearts wat sy ding vanuit die stad doen nie. As die meerderheid veeartse 'n ekonomiese en gerieflike bestaan in die stad wil maak, lyk dit of geen instansie hom anders sal oortuig nie. Met die deeglike en duur opleiding van die veearts is dit net logies dat hy op diè plek sal werk wat die meeste geriewe en beste inkomste bied.

Stelling 4 – 'n Boer maak 'n plan en 'n plan is 'n boerdery

Hierdie stelling is eintlik alreeds 'n spreekwoord. Hierdie waarheid is egter 'n indirekte faktor wat 'n rol speel in die afwesigheid van groot getalle veeartse op die platteland.

Dit is so dat die boer 'n vindingryke persoon is. Weens sy pioniersdae toe hy alles van vooraf moes prakseer en weens ver afstande, het hy geleer om homself te help. Hierdie eienskap word vandag weer verskerp met die al stygende petrolpryse en selfhelp het 'n inherente eienskap van boerdery geword.

Hierdie eienskap is in der waarheid 'n baie lofwaardige eienskap. Dit is egter nou nie onverwags dat die boer hom ook self sal wil help t.o.v. sy veesiektes, veemedisyne, entings, beplanning, kalfverlossing, ens nie. Noem dit en die boer probeer dit graag self. Stories loop selfs rond dat sekere boere heel goeie "chirurge" uitmaak. Hierdie benadering is nie net in kompetisie met die funksie van die veearts nie, maar in sekere gevalle kan dit selfs 'n verdringende effek hê, veral as stelling 2 in ag geneem word, waar die eienaar die een is wat die dienste aanvra. Die boer lewer egter nou 'n hele aantal dienste self.

Sommige boere vertel graag wat hulle reggekry het sonder die veearts se hulp en sonder onkostes. Andersins word weer vertel hoeveel die boer moes betaal vir veeartsenydienste terwyl die behandeling onsuksesvol was. Dit is menslik en vergeefbaar. As die veearts egter sy volledige funksie op die platteland wil vervul, sal die boer sy "probeer-en-fouteer" moet uitskakel. Die veearts sal dan meer dikwels en vroeër uitgeroep word. Meer veemedisyne sal onder die veearts se hantering geplaas moet word.

Die veearts kan ook help met beplanning en daar kan na meer diere d.m.v. die kliënt gekyk word. Werk sal vermeerder en die sukses en beeld van die veearts sal drasties verbeter. Die vraag is egter: wie gaan die ekstra onkoste vir die ekstra dienste moet betaal? Daar kan verskillende moontlikhede wees maar na ons mening sal dit beslis nie die veearts wees wat die dienste lewer nie. Dit skyn of slegs die bo-gemiddelde boer gereëlde veeartsdienste kan bekostig.

Daar is egter faktore wat bydra tot die boer se selfhelp-benadering. Een probleem kan lê by die voorligting van boere en voornemende boere. Daar word op soveel maniere klokkennis aangebied en tot op so 'n vlak dat die boere oortuig is hy hoor die klok lui. So dikwels moet hy te laat uitvind dat hy wat die gevorderde veeartsenykunde betref, nog nie weet waar die klepel hang nie.

Aan die boer word so dikwels die kitsresep aangebied, die oppervlakkige kennis teenoor die diepgaande en deeglike studie van die veearts. Die probleem is dat as die boer en die veearts net met die tipiese eindsimptome van 'n siekte te doen kry, beide se resultate dieselfde mag lyk. Die veearts weet egter alles van die siekte, die draers, die oorsakende parasiet, die atipiese simptome, die nuutste behandeling en variasies, die newe-effekte, prognose, ens. Die boer gee summier op eindsimptome dalk selfs 'n outydse middel en die dier word gesond, ten spyte van die behandeling. Op 'n afstand beskou lyk die diepgaande studie van die veearts nutteloos. Die feit is egter dat die biologie hom nie meganies laat hanteer nie. Daarvoor is al die diepgaande studie en agtergrond nodig.

Nou is die vraag: hoeveel dra oppervlakkige inligting wat aan die boer voorsien word by tot die probleem? 'n Mens wil nie inligting oor veesiektes van die boer totaal weerhou nie maar met die boer-maak-'n-plan eienskap, gee die inligting die boer net genoeg moed om veearts te speel tot 'n punt waar dit homself tot nadeel kan strek en waar dit 'n verleentheid vir die veearts word.

'n Ander faktor kan moontlik die veearts self wees. Die veearts is by uitstek die persoon om voorligting te gee maar die saak kan te ver gevoer word. Praktiese oorwegings, vêr afstande en min tyd kan daartoe lei dat ons selfs die boer aanmoedig om self 'n plan te maak en homself te help.

Ongelukkig skyn hierdie toedrag van sake onvermydelik te wees, maar bly 'n faktor wat nie onderskat moet word as die nodigheid van veeartsdienste in die platteland uitgebrei moet word nie. Te veel telefoon-konsultasie en opleiding van die boer kan die werklike aanvraag van veeartswerk verminder. Al is die veegetalle nou hoe groot, sal die veearts vind dat sy teenwoordigheid by gevalle minder benodig word en dit kan weer 'n rol speel om veeartse uit die platteland te weer.

Baie kollegas sal graag die selfhelp van die boer slegs wou toeskryf aan 'n veeartstekort. Ons stem saam dat dit ook 'n bydraende faktore kan wees, maar of groot getalle veeartse in die platteland hierdie inherente eienskap van "'n boer-maak-'n-plan" sal kanselleer, moet eers gesien word.

Om saam te vat

1. Die trek na die stad is aan die gang waarvan die patroon van vestiging van die veearts slegs 'n onderdeel uitmaak.

2. Al is daar baie vee op die platteland, word die vee slegs via 'n eienaar bedien. Dus die hoeveelheid dienste hang dus nou saam met die menslike behoefte om veeartsdienste aan te wend, ongeag dieregetalle.
3. Op die ou end staan dit nog die gekwalifiseerde veearts vry om sy besondere belangstelling te volg en om sy dienste te lewer waar hy wil.
4. Die boer moet weer afhanklik gemaak word aan veeartsenydienste as meer veeartse op die platteland wil vestig.

Ons gevolgtrekking is dus dat die oproepe, verklarings en selfs beskuldigings om die veearts op die platteland te kry nie altyd bg. stellings in gedagte hou nie. As ons nou weer na ons titel kyk, kan ons met vrymoedigheid sê dat die stad vir die veearts 'n groot geleentheid is. Die ontvolking van die platteland is 'n probleem waarvoor ons nie volstruis-politiek kan toepas nie. Die veeartsberoep moet verstedeliking in gedagte hou en daarby aanpas, die uitdaging aanvaar en op sy manier op alle terreine sy bydrae doeltreffender maak. Gelukkig hoef hy nie in die proses die veebedryf af te skeep nie.

Daar sal nog veeartse op die platteland vestig en werk, maar vanuit stede sal die boerdery ook bedien word, dalk sonder dat enige noemenswaardige verskuiwing platteland toe hoef plaas te vind. Alleeen op die wyse kan die veearts ook in 'n kompeterende posisie met ander professies staan, wat hulle almal toespits op stede.

Die veearts lewer vandag, en sal in die toekoms, sy bydrae lewer waar hy hom ook al vestig, want daarvoor is hy opgelei.

J. S. J. ODENDAAL, BSc., BVSc.

Olive Grinterrylaan 2, Fichardtpark, Bloemfontein 9301

LETTER TO THE EDITOR — AAN DIE REDAKSIE

OVERDOSING WITH PARBENDAZOLE AS A CAUSE OF PARALYSED LAMBS

Since 1968 intermittent complaints of paralysed lambs were received from various parts of South Africa. Investigation of this phenomenon revealed that on some farms in addition to paralysed lambs, lambs were born with various skeletal malformations. Paralysed lambs were unable to stand although no skeletal malformations were present. As they were unable to suckle, affected lambs usually died of starvation; if bottle fed, however, they survived but did not recover.

It is known that parbendazole can cause various skeletal malformations and other teratological malformations in lambs when ewes are treated with the drug during the first four weeks of pregnancy but no mention is made of paralysis (Lemon & Hancock, 1974; Middleton, Plant, Walker, Dixon & Johns, 1974; Shone, Philip & Fricker, 1974). A careful review of the history on some of these farms revealed the use of parbendazole during various stages of gestation. Although exact breeding data were not always available it appeared that ewes giving birth to paralysed lambs had been treated with parbendazole between approximately 23–62 days of gestation.

An experiment was undertaken to determine whether an overdose of parbendazole could cause paralysis in lambs. A group of 250 two tooth Merino ewes, 50 of which were used as controls, were treated with parbendazole at 180 mg/kg (normal dose 30–60 mg/kg) at various stages of gestation. Oestrus was synchronized with Estrumate® (Chloprostenol) and P M S followed by hand servicing. From the treated ewes a total of 68 lambs were born, 6 of which showed the paralysed lamb syndrome. Gross examination of these 6 lambs showed slight skeletal deformities in one and partial collapse of the cerebral hemispheres (hydrocephalus) in 2, one of which also showed cerebellar hypoplasia. Apart from unilateral renal aplasia in one, no other gross abnormalities were observed in the other three paralysed lambs. Paralysed lambs were born of ewes treated with parbendazole at 27, 31, 33, 38 and 54 days of gestation. Three ewes treated with parbendazole during the first 4 weeks of gestation gave birth to lambs with skeletal deformities. No abnormalities were observed in 18 lambs born from ewes in the control group.

Serological examination of the dams in cases of lame lambs or lambs with congenital deformities were negative for bluetongue, Rift Valley fever, Wesselsbron disease, Akabane disease and mucosal disease. No virus could be isolated from tissue of affected lambs submitted for virological examination.

From these results it is deduced that apart from skeletal malformations in lambs of ewes treated during the first four weeks of pregnancy, parbendazole at a high dosage rate could give rise to the birth of paralysed lambs when the ewes are treated as late as 54 days of gestation.

Results of the experiment will be reported in an article.

L PROZESKY & J P J JOUBERT

Onderstepoort Veterinary Research Institute
0110

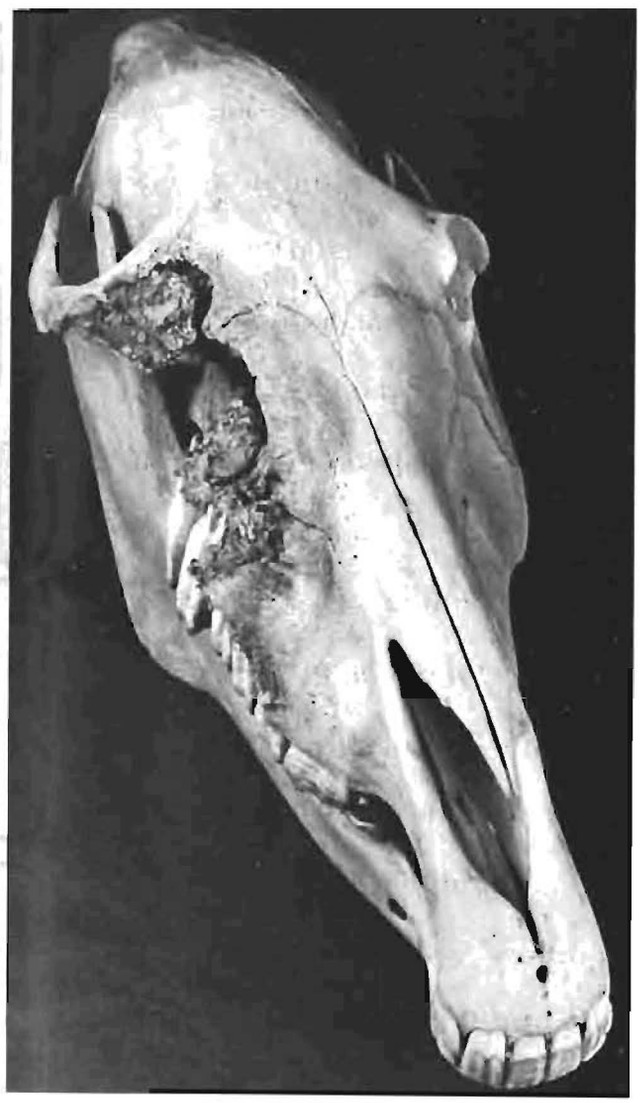
INFORMATION — INLIGTING

EDUCATION FOR RESEARCH IN SCHOOLS OF VETERINARY MEDICINE

The following paragraph was taken from a book by Dr Calvin W Schwabe entitled "Cattle, Priests, and Progress in Medicine" (p. 218). These thoughts are reproduced here in the interest of stimulating continued debate concerning the past, present and future relationship of veterinary medicine to human health. Interested readers are referred to the original text for more complete development of Dr Schwabe's ideas:

"Although by definition, veterinary medicine is an extremely broad field, it has remained a very small profession. The world's approximately 200 000 veterinarians are spread almost impossibly thin over an immense field for potential veterinary contributions. There are at least 4 known animal species for each veterinarian. As a consequence, in very few areas of its overall societal responsibilities does the world's tiny veterinary profession possess anything approaching enough depth. Almost all veterinarians everywhere are, of necessity, so fully involved day to day with the exigencies of the *particular* that they almost completely neglect the *general*. Thus veterinary medicine, as frequently perceived today, both within and without the profession, is much less than the sum of its existing parts. Since a harassed busyness has also characterized veterinary education, it is not surprising that veterinary schools fail to visualize fully their educational objectives in terms of constant and meaningful articulation of well-defined social goals. Consequently, veterinary schools have not yet met their responsibility to provide the varieties of specialized training required if individual veterinarians in greater numbers are to help meet more effectively the great challenges facing mankind. Today, therefore, it is not entirely a question of how many veterinarians there are but of what the schools train them to do. The first veterinary schools were created by governments precisely for the purpose of meeting some of the most critically felt social needs of the time. Over the years, social objectives of veterinary medicine have been partly lost sight of. As a result, although veterinary schools are already much more important to man than the public generally realizes, they have not yet become as important to society as they readily could."

FEATURE PAGE **TREFFERBLAD**



SQUAMOUS CELL CARCINOMA IN A HORSE

In January 1978 a Welsh pony mare, aged 5 years, was admitted with a neglected and advanced squamous cell carcinoma of the right eye and eyelids.

The eye was successfully enucleated with the orbit appearing to be normal at the time of operation.

According to the owner, some months later, sinuses broke out in front and below the orbit. In October the animal was admitted once again and after a radiological examination it was euthanased.

The picture shows the skull with bone destruction of the orbit and its surrounding structures.

Submitted by: Dr S W Petrick, Department of Surgery, Faculty of Veterinary Science, University of Pretoria.

PLAVEISELKARSINOOM IN 'N PERD

Gedurende Januarie 1978 is 'n Walliese ponie merrie van ongeveer 5 jaar ouderdom ingebring met 'n verwaarloosde en gevorderde plaveiselkarsinoom van die regter oog en ooglede.

'n Suksesvolle enukleasie is gedoen en die oogkas het tydens die operasie normaal voorgekom.

Enkele maande later volgens die eienaar, het sinusse uitgebreek voor en onder die oogkas. In Oktober is die perd weer ingebring en na 'n radiologiese ondersoek is die genadedood toegedien.

Die foto toon die skedel met die beenvernietiging in en om die oogkas.

Ingestuur deur: Dr S W Petrick, Departement Chirurgie, Fakultiet Veeartsenykunde, Universiteit van Pretoria.