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Volledige betroubare en beproefde reeks vir beeste en skape. Maklik om te gebruik. Maklik om te bekostig. Sekerheid vir die veearts wat plotbewoners bedien.

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DYLOX INSPUITBAAR Rondewurmmiddel vir beeste.
TRAMISOL Rondewurmmiddel vir beeste en skape.
SULPHAMEZATHINE 16% Middel vir koksidiose kalwers, lammers, hoenders en konyne.
OXY-VET Bree spectrum antibiotikum.
BACDIP NF2 Dipstof vir beeste, skape, honde en perde.
BACDIP AEROSOL Handige spuit vir lastige bosluis kolle.
DELTRAM Rondewurmmiddel vir skape en bokke.
LINTEX M Dood lintwurms en onvolwasse peervormige slak in kalwers en lammers.
BABESAN Rooiwater middel vir beeste.
TIGUVON SPOT-ON Dood luise op beeste.
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DIE EFFEK VAN BYTSODABEHANDELDE RUVOERE BY PERDE. I. BEHANDELDE LUSERNOOI AS BESTANDDEEL VAN 'N VOLLEDIGE RANTSOEN BY VULLENS.

H.P. VAN NIEKERK en S. COUVARAS

ABSTRACT: Van Niekerk H.P.; Couvaras S. **The effect of sodium hydroxide-treated roughages in horses: I. Treated lucerne hay as a constituent of a complete ration for foals.** *Journal South African Veterinary Association* (1979) **50** No. 2, 59, Dept. of Zootechnology, Faculty Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

The possibility of including sodium hydroxide-treated lucerne hay as a constituent in rations for horses was investigated by measuring its effect on the performance and certain bloodcomponents of growing foals. As no adverse effects were found it is concluded that further investigation is necessary to see whether the inclusion of low grade-treated roughages in the ration of horses would be of economic significance.

INLEIDING

Bytsodabehandeling van laegraadse ruvoere om die beskikbare energie-inhoud te verhoog deur verhoging van die ruvoer se verteerbaarheid is reeds so vroeg as 1919 deur Beckmann[1] toegepas, maar die plasing van hierdie tegniekvan voerprosesering op 'n meer ekonomiese en praktiese grondslag vir dierevoeding het eers betreklik onlangs geskied[2 6 8 10]. In Suid-Afrika het Hofmeyr & Jansen[5], Meissner, Frank & Hofmeyr[7] en Vosloo & Burger[9] die praktiese toepassing van hierdie tegniek op plase deur boere van stapel gestuur deur die ondersoek van o.a. die moontliheid van bytsodabehandelde ruvoere vir veeproduksie asook na die tegnieke van prosessering.

Daar die perd die vermoë besit om van growwe ruvoer gebruik te maak – alhoewel nie tot dieselfde mate as die bees, skaap of bok nie – en omdat sover vasgestel kon word geen navorsing oor die effek of verbruik van bytsodabehandelde ruvoere by perde gedoen is nie, is die doel van die huidige studie dan die volgende: Eerstens, om die aanvaarbaarheid van bytsodabehandelde ruvoere by perde vas te stel; tweedens, die invloed daarvan op die prestasie van groeiende vullens (soos gemeet deur hul vrywillige voerinnames en daaglikse massa toenames) te bepaal; en derdens, die effek daarvan op die uiteindelike bloedbeeld van die perde soos gemeet deur veranderings in sekere bloedkomponente. Antwoorde op hierdie en ander soortgelyke vrae is van die grootste belang vir beide die veearts en die perdebedryf – veral nou dat hierdie relatiewe nuwe veld van voerprosessering reeds hier in die RSA toegepas word.

PROSEDURE

Ses Nooitgedacht vullens (vier Nooitgedacht X Arabier en twee Nooitgedacht F2) met 'n gemiddelde ouderdom van tien maande is ewekansig in twee groep van drie elk en met 'n gemiddelde beginmassa van 250 kg vir elke groep ingedeel. 'n Volledig gebalanseerde rantsoen om aan die voedingsbehoeftes van hierdie vullens te voldoen is aan die hand van NRC se aanbevelings saamgestel. Die bestanddele en samestelling van hierdie rantsoen word in Tabel 1 aangegee. (NRC se aanbevelings vir perde 6–12 maande oud verskyn tussen hakkies in dieselfde tabel). Elke vul in albei groepe is afsonderlik gevoer.

Die lusernhooi in die rantsoen van die eksperimentele groep is vooraf met 'n 4 % bytsoda-oplossing behandel (droë proses). Die rantsoene vir beide die kontrole- en eksperimentele groep is *ad. lib.* aangebied.

Tabel 1: BESTANDDELE EN SAMESTELLING VAN DIE RANTSOEN MET DIE ONBEHANDELDE LUSERNHOOI. (Waardes uitgedruk op 90% droë materie basis)

Bestanddeel	Saamgestelde rantsoen	NRC se aanbevelings vir perde 6–12 mnde oud
Lusernhooi (grof gemaal)	50 kg	–
Geelmieliemeel (grof gemaal)	45 kg	–
Vismeel	5 kg	–
Mononatriumfosfaat	0,75 kg	–
Ruproteïen (%)	14,3	(14,0)
Verteerbare energie (mJ VE/Kg)	12,05	(12,0–11,5)
Ruvesel (%)	16,8	(10–20)
Kalsium (%)	0,74	(0,7–0,6)
Fosfor (%)	0,49	(0,45–0,35)
Ca:P	1,52:1	(1,5:1)

Tydens 'n aanpassingsperiode van twee weke het beide groepe egter die onbehandelde lusernooi in hul rantsoen ontvang. Voerinnames is aan die einde van die ses week proefperiode bepaal terwyl gewigstoenames elke 7 dae aangeteken is. Op die 42ste dag is bloed van elke vul getrek vir hematologiese ondersoek en elektroliet bepalings. Waargenome verskille tussen die gemiddeldes van die twee groepe is met behulp van 'n variansie analiese getoets[3].

RESULTATE EN BESPREKING

Die resultate van vullens gevoer op die onbehandelde en behandelde lusernhooi as bestanddeel van hul daaglikse rantsoen word in Tabel 2 aangegee, terwyl Figuur 1 die twee groepe se weeklikse massatoenames aandui. Tabel 3 toon die resultate van die hematologiese ondersoek en elektroliet bepalings.

Alhoewel dit uit die resultate van Tabel 1 blyk dat voerinnames by die eksperimentele groep effens hoër is (254,7 kg teenoor 234,5 kg), is die verskil, as gevolg van die min herhalings, nie betekenisvol nie. Massatoenames het ook nie betekenisvol verskil nie. 'n Analise van die resultate van die hematologiese ondersoek en elektroliet bepalings soos in Tabel 3 verskyn het eweeens getoon dat die verskille nie betekenisvol is nie, alhoewel dit SIP waardes van die eksperimentele groep die enigste is wat blyk effens laer te wees as die kontrole groep (1,36 mmol/l teenoor 1,61 mmol/l).

Uit die noue ooreenstemming in massatoename en voerinname tussen die twee groepe blyk dit asof die bytsodabehandelde lusernooi in die rantsoen van die

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Tabel 2: PRESTASIE VAN VULLENS GEVOER MET ONBEHANDELDE EN BYTSODABEHANDELDE LUSERNHOOI AS DEEL VAN HUL DAAGLIKSE RANTSOEN

Item	Kontrole Groep Gem.	±S.A.	K.V.(%)	Eksperimentele Groep Gem.	±S.A.	K.V. (%)	F-waarde 1,4 vg	
Getal diere	3			3				
Voerperiode (dae)	42			42				
Beginmassa (kg)	250	12,17	4,87	250	29,82	11,93	0,0	NB
Eindmassa (kg)	281,3	12,22	4,34	281,0	30,61	10,89	0,0003	NB
Totale massatoename(kg)	31,3	3,06	9,75	31,0	4,58	14,78	0,1	NB
Droë materie inname (kg)	234,5	25,67	10,95	254,7	31,94	12,54	0,73	NB

SB = Statistiese betekenisvol
NB = Statisties nie-betekenisvol

Tabel 3: HEMATOLOGIE EN ELEKTROLIET WAARDES

Hematologie:	Kontrolegroep Gem.	± S.A.	K.V. (%)	Eksperimentelegroep Gem.	± S.A.	K.V. (%)	F-waarde 1,4 vg	
Hb (g/l)	120	11,72	9,74	115	2,0	1,74	0,6	NB
RCC (x 10^{12}/l)	8,36	1,11	13,25	7,92	0,28	3,58	0,44	NB
Ht	0,33	0,03	10,50	0,31	0,02	5,59	0,8	NB
MCV (fl)	39,1	1,53	3,85	39,3	0,58	1,47	0,12	NB
WCC (x 10^{9}/l)	15,1	1,23	8,19	17,1	1,45	8,5	3,31	NB
Elektroliete:								
Ca (mmol/l)	2,75	0,07	2,37	2,69	0,10	3,86	0,64	NB
Mg (mmol/l)	0,67	0,05	6,66	0,76	0,09	11,55	1,99	NB
SIP (mmol/l)	1,61	0,22	13,48	1,36	0,03	2,35	3,69	NB
Na (mmol/l)	138,2	1,80	1,30	139,4	1,79	1,28	0,6	NB
K (mmol/l)	3,8	0,17	4,56	4,2	0,40	9,70	2,09	NB

SB = Statisties betekenisvol
NB = Statisties nie-betekenisvol

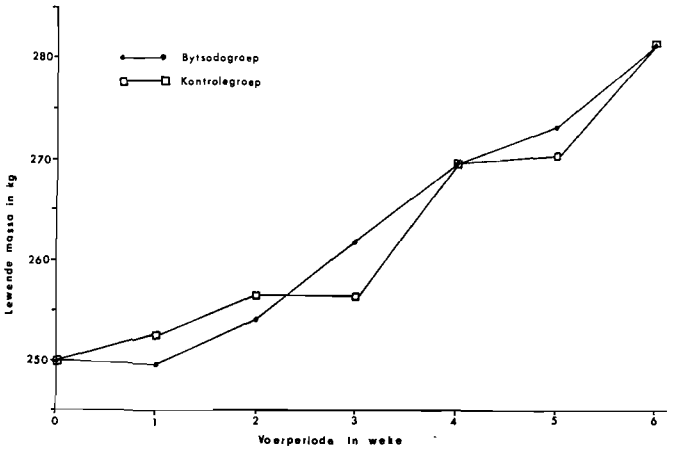

Fig. 1. Groeikurwes vir die kontrole- en bytsodagroep

eksperimentele groep net so aanvaarbaar is as die onbehandelde lusernhooi. Dat daar nie 'n noemenswaardige verskil in massatoename met die bytsodabehandelde lusernhooi as bestanddeel in die rantsoen van die eksperimentele groep is nie, was te verwagte en is in ooreenstemming met die *in vitro* bepalings van verteerbaarheid van verskillende ruvoere deur Hofmeyr[4], naamlik, dat bytsodabehandeling die verteerbaarheid van peulgewashooie nie tot dieselfde mate verhoog as nie-peulgewashooie nie. Die verteerbaarheid van lusernhooi het inteendeel onveranderd gebly.

As gevolg van die resultate verkry uit hierdie studie met betrekking tot die aanvaarbaarheid van bytsodabehandelde hooi asook met die feit dat daar skynbaar geen toksies uitwerking van sulke hooi op die perde is nie, word verdere studies met ander laegraadse ruvoere soos byvoorbeeld koringstrooi, hawerhooi, ens. (wat tot 60% verhogings in *in vitro* verteerbaarheid toon met behandeling), beplan. Die ekonomiese implikasies hiervan is voor die handliggend.

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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. 2, 61, (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 hypertropic 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 locomotory 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]
tionally, 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 if 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 correct. The symptoms disappear and the excess bone is gradually resorbed[1 22]. Retardation of the growth of

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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 extén-ding from the radial bowing 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 oesteodystrophy 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 the mineralized trabeculae[16 23]. If the blood supply to

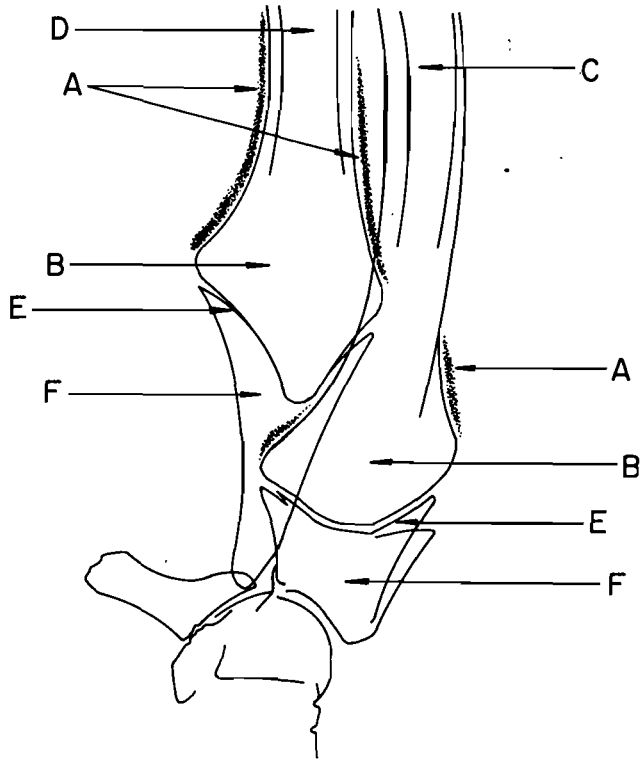

Fig. 1. Diagram of the lateral radiograph of the carpal findings in hypertrophic osteodystrophy of moderate severity. Extensive periosteal new bone formation extends from the metaphyses of both the radius and ulna, to surround the distal shafts of the bones (A). These cuffs of lace-like bone are separated 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).

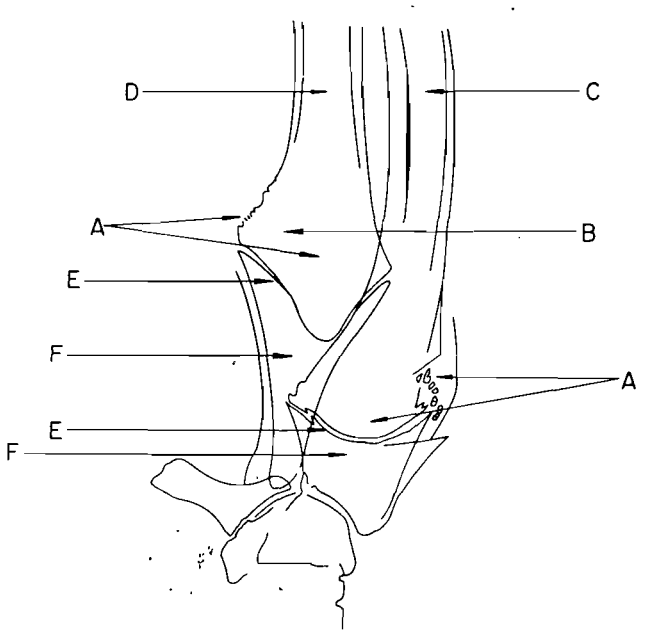

Fig. 2. Diagram of the lateral radiograph of the carpal findings in osteodystrophy II. Enlargement of the metaphyses resulting in flaring, and, the uneven granular appearance of the disorganized trabeculae causing cortical irregularity (A). Radiolucent areas in the metaphysis (B). Diaphysis of the radius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

the 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 retation 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. Radiographicall (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 aid 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 usuallyprovides 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

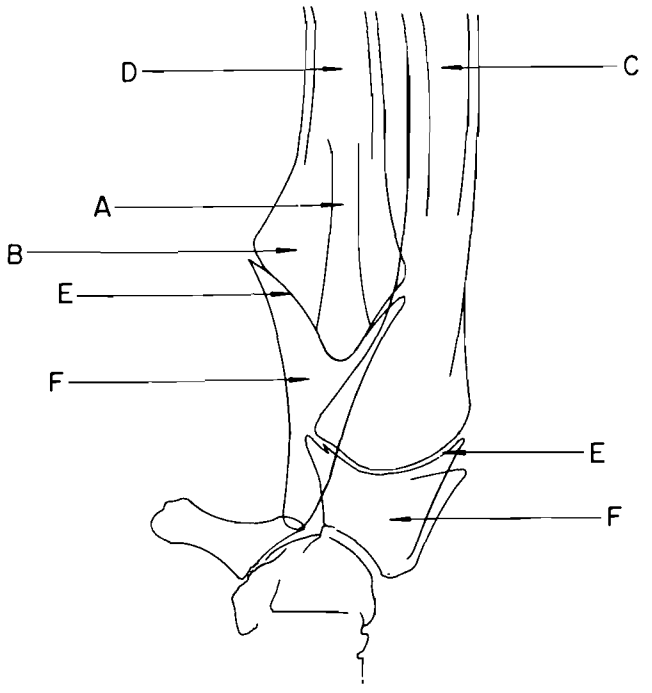

Fig. 3. Diagram of the lateral radiograph of the carpal findings in retained enchondral cartilage cores. Radiolucent (dark on a radiograph) strip extending from the growth plate, through the metaphysis, into the shaft of the ulna (A). The ulnar metaphysis is of normal size for a growing dog and has an 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).

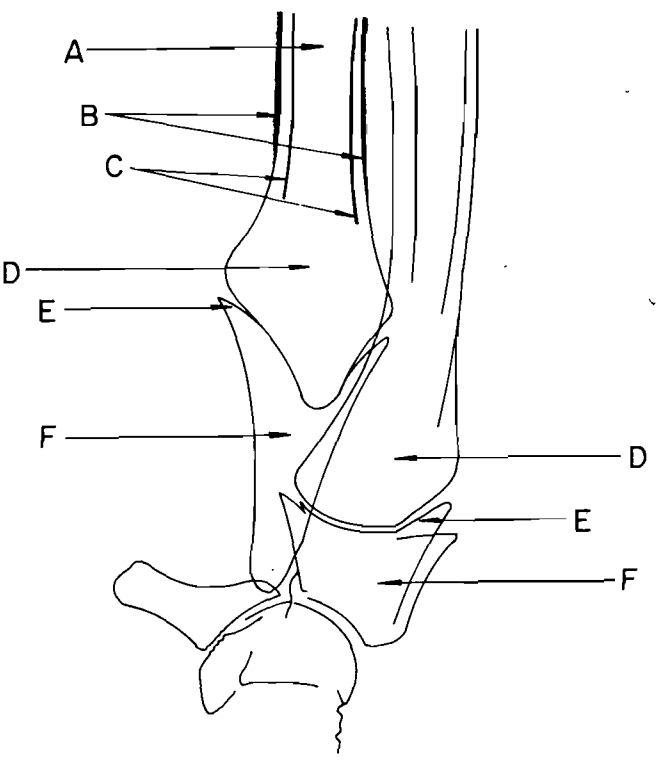

Fig. 4. Diagram of the lateral radiograph of the carpal findings in the middle phase of panosteitis. Diaphysis of the ulna has a patchy increase in density of the medullary cavity (A). There is also periosteal (B) and endosteal (C) new bone formation which results in accentuation of these lines. Normal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

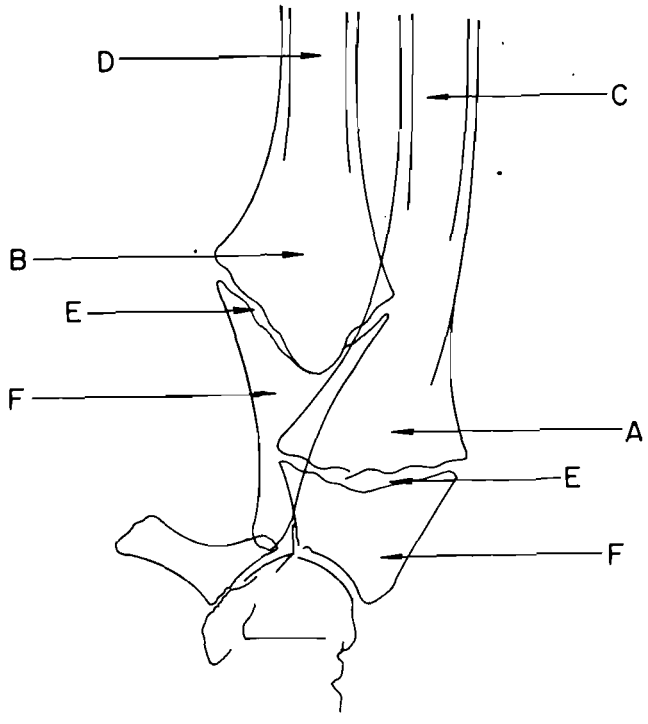

Fig. 5. Diagram of the lateral radiograph of the carpal findings in nutritional secondary hyperparathyroidism. Pathological folding fracture in the distal radial metaphysis (A). Demineralized metaphyses (B). The diaphyses of the radius (C), and the ulna (D) have very thin cortices a
Normal growth plates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

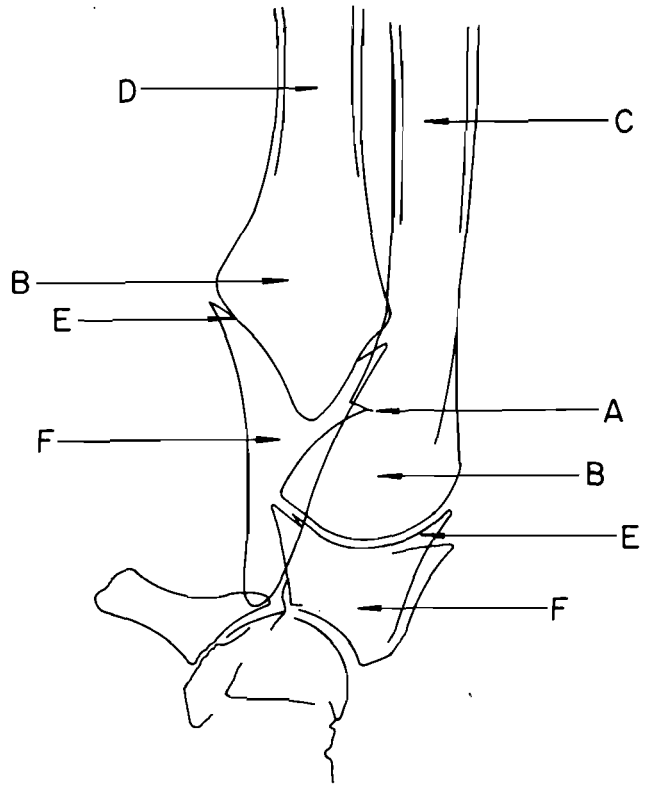

Fig. 6. Diagram of the lateral radiograph of the carpal findings in rickets. Radial (A) and ulnar (B) metaphyses are radiolucent and markedly enlarged, especially adjacent to the epiphyseal lines. The radial diaphyses is inadequately mineralized and is bowed cranialward (C). The diaphysis of the ulna 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-

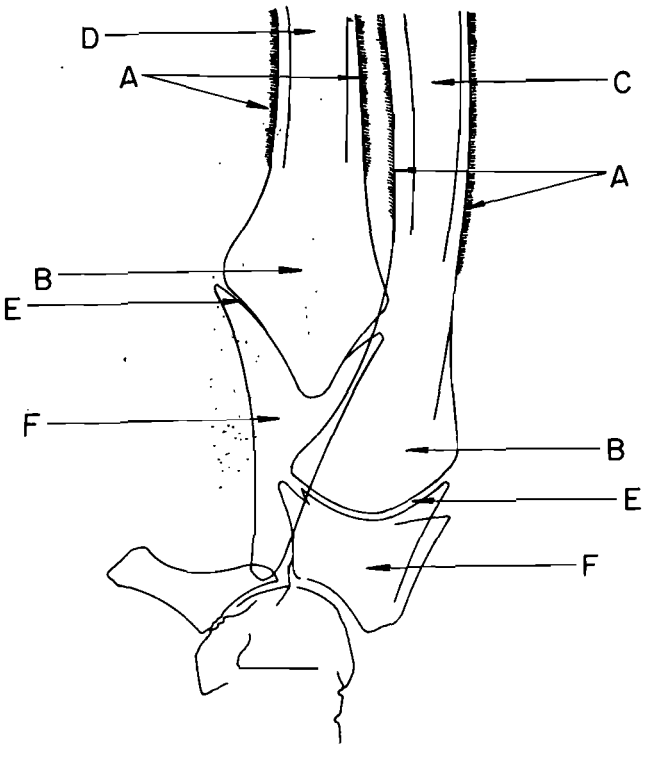

Fig. 7. Diagram of the lateral radiograph of the carpal findings in advanced hypertrophic pulmonary osteoarthropathy. Periosteal new bone formation occurs on the shafts of bones (A). The metaphyses appear normal (B). Radial (C) and ulnar (D) diaphyses are normally mineralized but have periosteal proliferations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

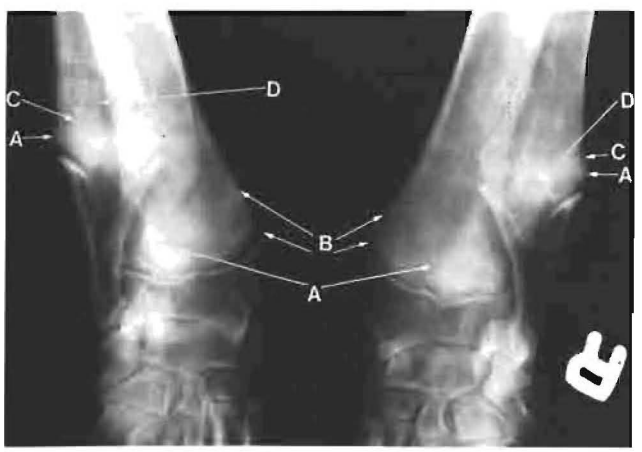

Fig. 8. Dorsopalmar radiograph of the left and right (R) carpal joints. In both forelegs the distal metaphyses of the radius and ulna are slightly flared and have an irregular groundglass appearance (A). Irregularity of the cortex is present in the medial aspect of the distal radial metaphyses (B). A 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).

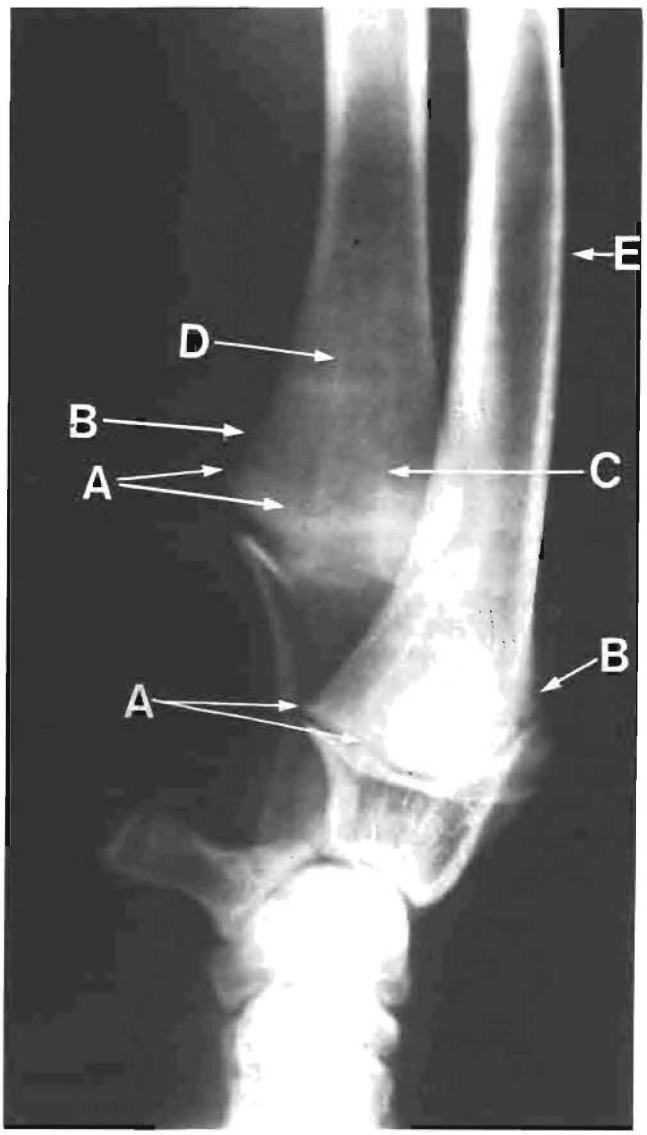

Fig. 9. Lateral radiograph of the left carpus. The distal metaphyses of the radius and ulna have an irregular groundglass appearance and are distinctly flared (A). Cortical irregularities are evident on the cranial border of the radial metaphysis and the caudal edge of the ulnar metaphysis (B). A radiolucent 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).

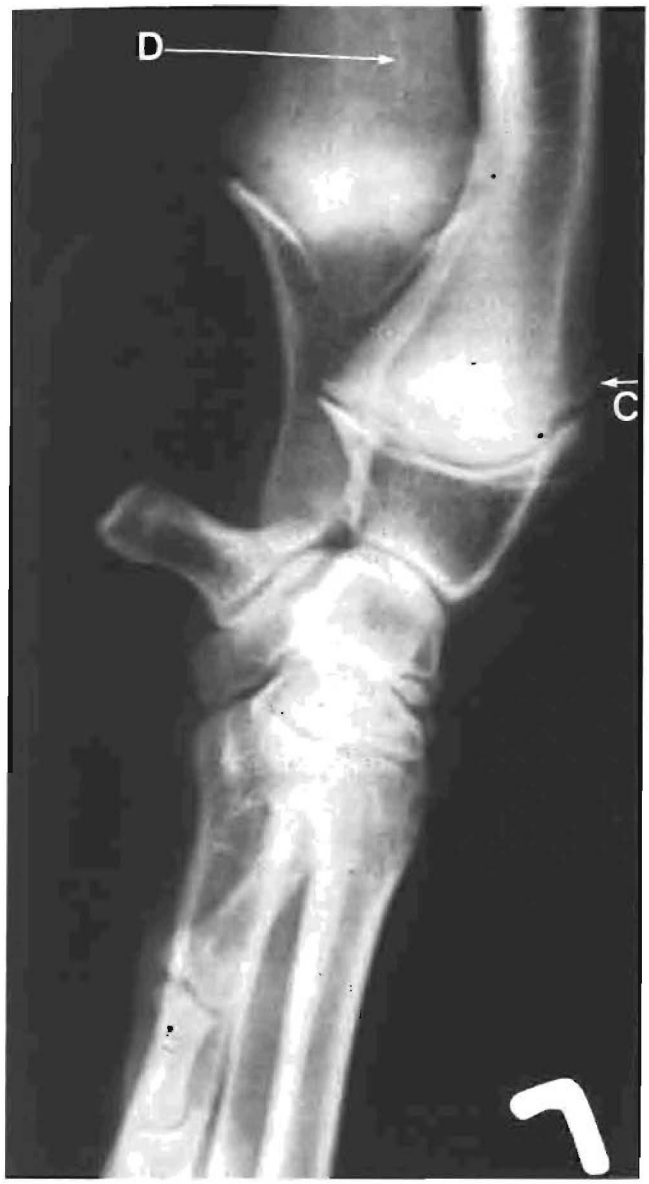

Fig. 10. Lateral radiograph of the left carpus taken 3 weeks after the initial examination. The irregularity of the radial metaphyseal cortex is less pronounced and has a smoother appearance (C). The enchondral cartilage core in the ulna is showing an increase in density (D).

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

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MULTIPLE SCLEROSIS RELATED TO ASSOCIATION WITH DOGS

Since 1977 investigators have reported a possible association between multiple sclerosis (MS) and contact with household pets. One of the most widely discussed reports appeared in Lancet (May 7, 1977) when investigators (Cook, Dowling, and associates) described 29 familial cases of MS and their close association with pet dogs in the 5 years prior to onset of their symptoms (see VPH Notes, September 1977).

This report was followed by a letter to the editor of the Journal of the American Medical Association in which a physician in New Jersey told of a higher incidence of exposure to household pets for 50 patients with MS than for 50 control patients.

In the February 1978 issue of the Annals of Neurology, Drs. Cook, Dowling, and associates report on further evidence of a possible association between house dogs and MS. This study was undertaken to determine whether a similar relationship between previous exposure to household pets and MS could be found for sporadic individual cases as had been seen for multiple family cases.

Sixty-one persons whose illnesses had been diagnosed as MS and an appropriate control for each composed the study population. These patients were obtained by referral from neurologists on the clinical faculty of the New Jersey Medical School, a hospital caring for patients with chronic MS, and local MS Society chapters.

Twenty-two patients were examined by the authors, and based on history, examination, and laboratory findings, the diagnosis was substantiated. A panel of board-certified neurologists agreed that findings in an additional 23 patients were pathognomonic for MS; these patients had remitting deficits of central origin in at least 2 separate anatomical locations. Of the remaining patients, 13 were excluded because the diagnosis of MS was not definite and 3 because they gave unreliable histories.

To do a case-control study the investigators asked each of the 45 patients for the name of a long-standing friend of the same sex and race who had lived in the general neighborhood prior to the MS patient's onset of symptoms. The 455 controls were thus of the same sex and race as their MS counterpart and generally of the same socioeconomic background. Matched pairs were all from urban/suburban New Jersey.

A detailed questionnaire on pet exposure was completed by the investigators in person or over the telephone with patients and controls. Participants were asked about ownership of dogs and cats, characteristics of the pets, and whether those pets had had distemper or had exhibited neurologic signs, such as convulsions, staggering, weakness, or paralysis. Indoor pets were defined as animals that slept in the house.

No difference in dog or small-dog ownership was found between patients and controls. However, a significant difference between the 2 groups became apparent when the dogs were characterized as indoor or outdoor pets. More MS patients than controls owned indoor dogs at any time before onset of MS symptoms and within 5 years before onset of MS.

Editorial Note: Results of the second study conducted by Cook, Dowling, and associates corraborated the findings of their earlier study, i.e., a statistical association between MS and ownership of dogs that sleep indoors. As a resultt, chance seems more remote as an explanation of the observations in their New Jersey patients.

However,it is possible that certain biases may still be inherent in the second study. Patients with serious disease of unknown cause are, often unconsciously, more aware of past exposures than healthy persons and even ready to ascribe their misfortune to the vividly recalled experience. Owners of indoor dogs may well differ from other persons in many respects, such as their degree of exposure to sunlight and other environmental factors, their travel and other social activity, or their occupations. Any of these are also plausible risk factors for MS. Finally, even if the association of small-dog ownership and subsequent MS were to prove genuine, the indoor dog might merely represent an indicator of some more relevant feature, such as the types of microorganisms carried by rodents or soil in homes where dogs sleep indoors.

The association between MS and indoor-dog ownership can only be confirmed by further carefully designed epidemiologic studies in other populations, and the causal connection will require still additional painstaking effort.

A SPECIFIC FORM OF ABOMASAL PHYTOBEZOAR IN GOATS AND SHEEP

G.F. BATH* and T. BERGH†

ABSTRACT: Bath G.F.; Bergh T. **A specific form of abomasal phytobezoar in goats and sheep.** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 69 (En) Regional Veterinary Laboratory, Private Bag X528, Middelburg (Cape) 5900. Rep. of South Africa.

A specific form of phytobezoar in goats and sheep is described with regard to epizootology, symptomatology, gross pathology, and gross morphology of the bezoars. The probable mode of formation and control measures are also discussed.

INTRODUCTION

Various types of fibrous concretions (bezoars) within the digestige tract of domestic animals have been described[2-6 8 12-15 17-21]. Some authorities do not mention them as disease entities[7 9 11 16] while others regard them as being of minor importance[8 13 19]. Trichobezoars (hairballs) are well known in cats[17 18] and young ruminants[5 8 13 14 18-21]. In cats their formaion is believed to be a result of fur ingested during normal grooming[17 18] or secondarily due to a skin irritation[17] while in young ruminants it is usually the result of a deficiency of natural food fibres in artificial diets[8] when animals lick themselves excessively and swallow loose hair in an apparent attempt to make good the deficiency. In other cases it is ascribed to hair licking secondary to skin irritation by external parasites[14] or a specific toxin in the plant *Chrysocoma tenuifolia*[20].

Phytobezoars (plant fibre balls) of several types and origins have been described[3-5 13 15 18 21] while trichophytobezoars (mixed plant-hair balls) have also been recorded[12 17]. Steyn[21], Louw & Steenkamp[15] and Banting[3] describe ovine phytobezoars resulting from the ingestion of bushmangrass *(Stipagrostis obtusa* and *S. ciliata,* formerly *Aristida uniplumis* and *A. brevifolia),* the seeds of which show a tendency to cling together and form balls. Siegmund[18] notes the formation of fibre balls on poor quality fibrous feeds, while Belschner[5] mentions felt balls which arise from the ingestion of certain shrubs and plants covered with fine hair. Unpublished findings in Southern Africa include those of Albl[2], who has seen phytobezoars formed by *S. ciliata* in the Namib and also the annual *Lotonis platycarpus* in the Kalahari.

Since 1973 several outbreaks of a specific form of abomasal phytobezoariasis of goats and sheep have been investigated in the Cape Midlands and surrounding areas[4]. More recently the condition has been observed in Gordonia where it seems to be a definite and extensive economic problem. For this reason a more complete description of the disease is thought necessary. Other aspects of investigation are to be published separately.

EPIZOOTOLOGY

Graphic distribution: In the Cape Midlands, the disease is largely confined to the more mountainous parts, although cases are occasionally seen in the plains. Sporadic cases are encountered, however, over a very wide area; the condition occurs throughout the upper Karoo and surrounding areas. In Gordonia and Kenhardt cases are seen in both Kalahari sand and broken country.

Vegetation and veld management: Occurrence of the bezoars is not limited to certain veld types but it does seem more common on those types found on mountains in the south. In the north the disease is associated with the plant communities which grow in the dune "streets" between sand dunes. It appears that bad veld management with consequent deterioration of the veld, limiting the variety of vetegation and increasing undesirable plants may lead to greater incidence of cases within a given region.

Climate: No definite conclusion could be reached concerning the possible role played by climate. Some farmers maintain that the condition is more prevalent in droughts, while others, on the contrary, report increases in rainy years.

Season: There seems to be a peak incidence in late winter and early spring, although cases may be encountered throughout the year.

Species and Breed: On most farms with both goats and sheep, goats are relatively far more susceptible, and Boer goats much more so than Angoras. Indeed in the initial stages it appeared that the condition was exclusively confined to Boer goats, but subsequently several cases in Angoras and occassional cases in sheep were seen. In Gordonia the condition is common in sheep but has not been recorded in goats. This is possibly partly because goats form a small auxiliary part of farming operations.

Sex and Age: There is no anomaly in distribution between the sexes. Fatal cases are far more common in goats between 3 and 15 months than in older or younger ones. The peak incidence of deaths seems to be between 6 and 12 months.

Condition: Animals are often thin by the time clinical symptoms are noted, but there is no evidence that poor (or good) condition is a necessary precondition for development of the disease.

Morbidity: On the average farm throughout the region, cases are virtually unknown. On affected farms generally morbidity is between 1–10 % of the total flock, but occasionally the figure in a specific flock may be 20 % or even higher. The true morbidity rate is difficult to assess since many animals are not clinically ill though bezoars are present and are found either by palpation or incidentally after slaughter.

Mortality: Many of those animals with bezoars eventually die as a direct or indirect result. A large number of fibre balls or one large ball may impede the flow of ingesta and render the animal susceptible to various dis-

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eases associated with gastrointestinal stasis. Frequently total obstruction, with or without abomasal or intestinal rupture, leads to more sudden death. This is usually found only when smaller bezoars are present.

SYMPTOMATOLOGY

In most cases the onset is insidious and characterised by a slow loss of condition, increasing depression and worsening appetite. Affected animals tend to lag behind the flock, or stand alone with the head held low in later stages. The abdomen often becomes enlarged and pendulous, and swings from wide to side as the animals run. The rumen contents are watery, intestinal movements are sluggish or absent, and faeces are often scanty and foul-smelling. On deep palpation caudal to the Siphoid cartilage, the presence of hard lumps within the abomasum can readily be felt. The best way of achieving this is to straddle the goat or sheep just ahead of the hind legs and clasp the hands together under the belly. Then by raising the hands and pressing them together, bezoars are usually easily felt. In young animals they are mostly small and often several in number, while in oder animals single or a few large structures are present.

Occasionally animals die very suddenly without any warning symptoms. Taking into account the extensive nature of farming in the area, however, often there is simply no one present to observe initial symptoms. True cases of sudden death are generally due to sudden complete blockage of the digestive tract, sometimes accompanied by rupture of the intestinal or gastric wall.

POST MORTEM EXAMINATION

Carcasses are usually emaciated to a variable degree, and seem to decompose rapidly. Apart from such nonspecific secondary changes as degenerative changes in the parenchymal organs, the only important lesions are confined to the abdomen and the gastro-intestinal tract. The rumen is usually thin-walled, and its papillae are poorly developed and sometimes covered by a fine mushy substance. The contents are watery, ingesta is usually finely ground, and there is a very characteristic stale, foul smell present. The colour of the ingesta is usually greyish-brown to almost black. No phytobezoars have been found in the rumen, reticulum and omasum. These last two structures show changes similar to those in the rumen.

The abdomasum is often much enlarged but the wall is thin. Frequently ulceration or frank rupture is present at places where bezoars touch the gastric wall. Abomasal contents are watery, blackish and foul-smelling. One or many bezoars may be present. Occasionally smaller bezoars pass the pyloric sphincter and lodge lower down in the small intestine, where once again obstruction, ulceration and rupture may occur. Below the bezoars the intestines are thin walled and often nearly empty. A low degree of inflammation is often visible, while after rupture there are, of course, all the signs of an acute peritonitis.

DESCRIPTION OF THE BEZOARS

Sizes: The smallest structures are only 10 mm in diameter, while the largest ones have an average diameter of 150 mm. Most however have a diameter of between 20–80 mm.

Mass: The smallest weigh about 0,5 g (dry mass) while the largest one seen, weighs just under 270 g. The smaller ones generally weigh between 2–10 g while the larger ones in older animals are usually between 20–100 g.

Consistency: The masses are always compact, dense and macroscopically have a fine velvety texture both on the outer surface and on section. When freshly removed from the abomasum they sink in water.

Colour: The great majority are buff to khaki in colour, though a few may be dark brown to almost black on the surface or in certain layers. Sometimes the surface shows patches of incomplete layers of different colours. The surface is nearly always dull, but occasionally it is shiny and even waxy in appearance.

Shapes: The surface is in most cases smooth and even, but sometimes irregular deep fissures may be present, often only on one aspect of the bezoar. The majority of bezoars are roughly spherical, but often vary towards egg shapes, ellipses and flattened forms. Many others have definite facets which may either be convex, concave or flat. Edges between these planes are always rounded off. These facetted structures form a large variety of shapes and may have 4, 5, 6 or (less commonly) more faces. In this way rounded pyramids, cubes and so forth are formed. Occasionally very large bezoars are seen which appear to be formed from several smaller structures. These, like the occasional bizzare forms encountered, are very difficult to describe. A selection of some of these shapes is presented in Figs. 1 and 2.

Fig. 1. Pappus hair phytobezoars. Scale in centimetres.

Structure: The bezoars are made up to innumerable very fine plant fibres which are densely packed together like felt. On macroscopic examination the size of individual fibres seems remarkably even, although on the outer surface much coarser fibres are often found loosely attached. On section there is a strong tendency to

Fig. 2. Pappus hair phytobezoars. Scale in centimetres.

Fig. 3. Cross-section of a small phytobezoar. Note even texture, layering and the absence of a nucleus.

layering visible (Fig. 3). In some bezoars the layers are even, while in others they are irregular. There is no sign in the central core of a difference in structure or of some object which could have served as a nucleus for growth.

PROBABLE MODE OF FORMATION

It is clear even from gross examination of these structures that they consist of plant fibres of some sort. The results of examinations are to be reported separately, but the appearance of individual fibres is very similar to the pappus hairs around the seeds of some common karoo bushes. These pappus hairs act as a parachute or sail to the ripe seed and aid their windborne spread.

Sheep and particularly goats often eat the flowering heads of karoo bushes[10], and it seems quite likely that they could eat sufficient quantities of the mature flowers of common karoo shrubs to allow for the formation of phytobezoars. In the case of the snowbush (*Eriocephalus* spp.) the mature seeds are inclined to clump together forming loose balls, and these if eaten by stock would constitute a very concentrated form of fibre. It appears that stock may be quite fond of eating these seeds. Certain ants carry karoo bush seeds back to the nest, and accumulations around the entrance would be a further concentrated source of fibre[1].

The remaining question is how the loose fibres are formed into the relatively dense mass of a bezoar. While initially it was thought that a substance which could cake the fibres together was responsible, it later became apparent that the fibres must felt together like wool by means of ultramicroscopic hooks or scales.

CONTROL

Treatment is presently confined to surgical removal of bezoars, using a described technique[3 6]. Obviously some as yet undiscovered substances which, if dosed, could break up these structures without damage to living tissue would be the treatment of choice. No convincing evidence of such a substance has yet been forthcoming. The bezoars seem to remain apparently unchanged in the abomasum for years; their chemical structure must therefore be fairly resistant. Treatment by dosing with purgatives or oily substances in an attempt to have the bezoars passed down the digestive tract, are useless, if not frankly dangerous. Similarly attempts to break the bezoars manually through the abdominal wall have proved to be of no value.

If the surmise is correct that these fibre balls are made up largely of the pappus hairs of certain Karoo bushes, then a certain degree of prevention is possible by manipulation of grazing management. Unfortunately the Karoo bushes under suspicion are often very common if not ubiquitous, and it is difficult to avoid them at any time. It must also be noted that numerous other Karoo bushes which have not been investigated as yet could also be partially responsible for the formation of bezoars – indeed, there is every likelihood that several species are collectively responsible. However, by avoiding camps with very large numbers of these bushes especially during and after flowering, some degree of diminution of the problem can be expected. In the long term veld management aimed at decreasing the relative numbers of responsible plants should be considered.

Apart from the surgical approach the owner can limit his economic losses to a considerable degree by palpating each animal in the flock for the presence of bezoars. Affected animals (which are often still in good condition) can then be sold for slaughter in a reasonable condition and some of their value realised.

DISCUSSION

We believe that this condition is one which was either previously unknown, or its true importance and cause not recognised. While an acute rupture or obstruction would hardly be missed by the veterinarian, this is not always true of the farmer who so often performs what clinical and post mortem examinations are done in these areas. Where bezoars are only a contributory factor to death, or are not related to death but have lowered production slightly, the chances of recognition are slight unless the abomasum is palpated or opened. This form of pappus hair phytobezoar can easily be differentiated from other types of locally occuring phytobezoars[3 15 21], and especially zootrichobezoars[20], purely on its gross appearance. No other local bezoar approaches the pappus hair bezoar in fineness of fibre or

evenness of texture, and they should be easily recognisably by the description given above.

It is suggested that these phytobezoars could be called pappus hair bezoars for scientific use, and that the name velvet stomach balls (Afrikaans: fluweel pensballe) be adopted for popular use.

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TOXOPLASMOSIS – GEORGIA

One of the largest outbreaks of toxoplasmosis ever reported in the United States occurred in patrons of a riding stable in Atlanta, Georgia, in October 1977. Thirty-seven persons became ill with a syndrome compatible with toxoplasmosis (fever, lymphadenopathy, and headache) or had serologic evidence of acute infection with elevated indirect flourescent antibody titers (IFA >1:4096 or IFA-IgM positive). An additional 47 persons who rode at the stable and were not ill were tested and were seronegative or had evidence of past infection. Twenty-one persons in the surrounding community, including 4 with illness compatible with toxoplasmosis, were evaluated and had evidence of past infection or were seronegative.

Forty-seven persons at 2 other stables in the area were interviewed and tested for antibody titers of *Toxoplasma.* None of them were or had been ill, and none had elevated titers.

Cats from the stable associated with the outbreak were bled, and serologic tests revealed that 2 of 3 cats had elevated *Toxoplasma* titers (1:256, 1:1024). Kittens born prior to the outbreak had *Toxoplasma* isolated from their tissues.

Rodents were obtained from 2 stables and tested by mouse inoculation for evidence of *Toxoplasma* infection. Of 4 mice tested from the stable where the outbreak occurred all have been positive. Test results on rodents from the other stable are pending. A cat that was fed mice produced oocysts.

Persons who spent most of their time at the west-end of the stables were found to be at greatest risk of infection. The attack rate was higher in those who went to the stable every day compared with those who went less often. The overall rate of clinical disease in this outbreak was extremely high (35 % of 37 seropositive persons). No common meal was served to persons who patronized the stable, and those who brought lunches did so individually.

Toxoplasmosis, a systemic protozoan disease caused by *Toxoplasma gondii,* has 3 known modes of transmission: from pregnant women to their fetuses, by eating poorly cooked or raw infected meat, and presumably from infected cats. Cats (and all *Felidae*), the only animals capable of excreting oocysts in their feces, generally excrete the cysts only at 1 time in their lives, and then only for a period of approximately 2 weeks.

Data collected to date suggest that cats were the source of infection for persons in this outbreak, with an aerosol in the enclosed arena or fingers-to-mouth transmission as the possible modes of exposure. These modes of transmission would explain how persons without direct exposure to cat feces or under-cooked meat might become infected with *Toxoplasma.*

Infection with *Toxoplasma* generally causes subclinical illness. Exposure to he disease is apparently quite common; 4 %–30 % of residents of the United States have serologic evidence of past infection.

A FIELD OUTBREAK OF SUSPECTED STACHYBOTRYOTOXICOSIS IN SHEEP

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ABSTRACT: Schneider D.J.; Marasas W.F.O.; Dale Kuys June C.; Kriek N.P.J.; Van Schalkwyk G.C. **A field outbreak of suspected Stachybotryotoxicosis in sheep.** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 73 (En) Veterinary Regional Laboratory, Private Bag X5020, Stellenbosch 7600, Rep. of South Africa.

An outbreak of mortality in a flock of mutton merino sheep in which 109 out of 568 sheep died in the south-western Cape Province, is described. It was characterized by haemorrhagic septicaemia, anaemia, leucocytopaenia and haemorrhagic tendencies. Mortalities followed unseasonal and heavy summer rain, extended over a period of 6 months and were associated with the uninterrupted consumption of sheep cubes processed on the farm from severely fungus-infested wheat, barley and rye straw for a period of at least one month. The main clinical signs occurred in two phases: an elevated body temperature, listlessness, epistaxis and intermittent haemorrhagic diarrhoea during the first phase of the outbreak, and a progressively worsening anaemia, leucocytopaenia and less severe haemorrhagic tendencies and a terminally elevated body temperature during the second phase. The predominant autopsy findings were purpuric haemorrhage on serosal and mucosal surfaces and in most of the organs, enterorrhagia and severe pulmonary congestion and oedema during the first stage; anaemia was the predominant sign during the second stage – widespread haemorrhage still occurred but was less extensive. *Pasteurella haemolytica* was isolated from most of the animals autopsied during the first stage. Histologically the most salient features were atrophy and necrosis of the lymphoid tissue, aplastic anaemia and a markedly impaired inflammatory response. Extensive post-natal lamb mortalities, probably due to an *Escherichia coli* infection precipitated by the toxicosis, occurred during the outbreak.

Toxigenic strains of *Stachybotrys chartarum* were isolated from the wheat and barley straw. Diethyl ether extracts of the wheat straw, sheep cubes and *S. chartarum* culture material elicited positive skin tests in rats following intradermal injection and the presence of 12,13-epoxytrichothecenes in these extracts were confirmed by thin layer chromatography. In feeding trials sheep cubes and wheat straw caused the death of 4/4 one-day-old Pekin ducklings and weanling Wistar rats in six and nine days, respectively.

This is the first description of an outbreak of disease in sheep associated with the ingestion of *S. chartarum*-infested food components in the Republic of South Africa.

LITERATURE REVIEW

Stachybotryotoxicosis is a mycotoxicosis of animals, particularly horses, and man caused by the ingestion of or contact with hay, feed or litter contaminated with toxic strains of the cosmopolitan saprophytic fungus referred to in the literature as *Stachybotrys atra* Corda or *S. alternans* Bon.[2 8 9 10 13 25]. The disease was first described during the early 1930's in the Ukrainian U.S.S.R. where it caused the death of thousands of horses and also affected farm labourers handling contaminated hay. Field cases of stachybotryotoxicosis have also been reported in cattle, chickens and zoo animals in the U.S.S.R.[8 9 13 25], and more recently in horses, calves, sheep, swine and poultry in a number of eastern European countries[2 3 13 20 21 25 28]. Stachybotryotoxicosis has been induced experimentally with either mouldy feed, toxic *Stachybotrys* cultures, or various extracts of these materials in the following animals: horses[8 9 11], cattle[11 22], sheep[3 11], poultry[19 21 23 26], and laboratory animals including mice, guinea pigs, rabbits and dogs[8 9 11 14 20].

The lesions of stachybotryotoxicosis resemble those caused by a group of sesquiterpenoid mycotoxins known as 12,13-epoxyΔ^9-trichothecenes and toxic strains of *S. atra* have recently been shown to produce several biologically active trichothecenes[5 6 25 28]. Three of these toxic compounds known as satratoxins have been chemically characterised, i.e. satratoxins C and D as the known fungal metabolites verrucarin J[7], and roridin E[6], respectively, and satratoxin H as a new macrocyclic ester of 12,13-epoxytrichothecene structurally similar to roridin E[7]. The complex nature of the toxins may contribute to the different types of stachybotryotoxicosis observed[13]. The trichothecenes, in general, are radiomimetic and affect rapidly dividing cells, i.e. those of the bone marrow, lymph nodes, intestinal mucosa and other organs[27]. Most of the trichothecenes have a skin irritating effect resulting in a marked inflammatory reaction and necrosis following local application to the shaved skin of rabbits. This dermal toxicity test has become a standard biological test for *Stachybotrys* toxins[6 8 9 10 11 15 20 25 28].

The fungus is found predominantly on cellulose-rich plant material and also in the upper layers of the soil. It has been isolated from wheat, oats, barley, rye, peas, cotton and sugar cane roots[13]. The toxins which are present in each part of the fungus as well as in the substrate, are not inactivated by gastric juices, X-ray and ultraviolet irradiation or heat (120°C for two hours). Fungus-infected food remains toxic for years. Animals appear to have a preference for *Stachybotrys*-infected mouldy straw possibly due to the flavour imparted by the fungus[2].

Depending on the mode of exposure and the relative toxicity of the involved material, the disease in animals may manifest as one of the following types: dermal involvement, general toxicosis, nervous form and abortions[13]. Some species are more prone to show signs of the one type than the other. Particularly in the general toxicosis, secondary bacterial infections play a major role in the course, clinical manifestation and outcome of the disease. This is ascribed to a marked immunosuppression involving both the cellular and humoral immune system as a consequence of the toxic action of the ingested toxins[1 2].

The major part of the Russian and eastern European literature reviewed by Forgacs[9] and Hintikka[13] pertains to the disease in horses in which the syndrome is best characterised. The disease is endemic, seasonal, appears after animals are stabled and disappears when they are returned to pasture. It occurs either as an outbreak with most cases appearing within two to three hours, or as sporadic deaths over a period of three to

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four months preceeding a general outbreak. Depending on the degree of toxicity of the ingested material and/or the rate of intake, the ensuing clinical signs may be classified as either a typical or atypical form.

The typical form follows ingestion of less toxic material over a prolonged period and is divided into three stages. In Stage One which lasts from 8–12 days and occasionally up to 30, buccal lesions are the most common clinical signs. They appear from 2–10 days following initial exposure and vary from slight exfoliation to superficial or deep necrosis at the mucocutaneous junction of the lips. Oedema, hyperaemia, necrosis and ulceration of the oral mucosa, excessive salivation, rhinitis, conjunctivitis, swollen maxillary lymph nodes and an elevated body temperature are additional signs. In spite of continuing ingestion of toxic material, these signs may subside after two weeks. Stage two is largely asymptomatic, lasts from 15–20 and occasionally up to 50 days and is characterised by an increasing leuco- and thrombocytopaenia, an increased clotting time and occasionally a total inability of the blood to clot. Stage three is characterised by a severe and progressive thrombo- and leukocytopaenia, it is commonly fatal and lasts from one to six days. Affected animals are depressed, have an elevated body temperature, secondary infected necrotic areas reappear on the buccal mucosa, and diarrhoea and septicaemias are common. Death may be rapid or prolonged. Pregnant mares abort shortly before death.

The atypical or shock form is not common, develops within 10–12 hours and up to 72 hours after ingestion of highly toxic material and may occur in individual animals or among animals afflicted with the typical form of the disease. Buccal lesions and blood abnormalities are absent while nervous signs (loss of reflex response, hyperaesthesia, hyperirritability and blindness) and signs of shock are the predominant clinical features. Additional signs are anorexia, difficulty in swallowing, an elevated body temperature, intestinal atony or hyperperistalsis and possibly haemorrhages in the mucous membranes. The agonal period is prolonged and accompanied by periodic spasms and tremors. Death appears to be due to respiratory failure.

At autopsy, changes found in the typical and atypical forms are essentially similar and are characterised by extensive haemorrhage and necrosis in many tissues particularly the subcutis and skeletal muscles, serous and mucous membranes and certain parenchymatous organs. Extensive suggillations and ecchymoses often resembling large bruises, are found on the costal pleura, diaphragm, mesentery and splenic capsule. Numerous haemorrhages also occur in the lymph nodes, lung, liver, adrenals, brain, spinal cord and meninges. The bone marrow is jelly-like.

Necrosis of the mucous membrane is observed along the entire gastro-intestinal tract. In the buccal cavity circular or ovoid, clearly outlined, yellowish-gray necrotic foci are found on the lips, soft palate and tonsils.The oesophagus is rarely affected while haemorrhage and necrosis and a catarrhal gastritis is evident in the stomach. While the small intestine is only affected by a catarrhal enteritis, the most typical changes are observed in the large intestine. In the latter, two basic lesions are observed: small yellowish-gray papules on the mucous membrane and deep-seated ulcers extending into the submucosa and muscular layer. A serohaemorrhagic enteritis may occur in some cases while areas of necrosis alternating with haemorrhage are seen in the more acute cases.

Varying degrees of degeneration ranging from hydropic degeneration to fatty changes are found in the liver, kidney and myocardium. The lungs are intensely congested, oedematous and commonly contain haemorrhages. Early bronchopneumonia is rare. In the typical form the adrenal cortex is atrophic and depleted of lipids while there is marked cortical haemorrhages in the peracute cases. Zones of necrosis are characteristically not limited by a reactive zone while the lymph nodes are virtually depleted of lymphocytes. The bone marrow may show focal areas of necrosis and a marked decrease in cells, particularly granulocytes.

Danko[2] and Hintikka[13] reviewed the most salient features in other domesticated animals. The disease in cattle resembles that in the equine although they are less susceptible, and the incidence is enhanced by the ingestion of straw or hay supplemented with carbohydrate-rich dietary components. Large outbreaks may occur while mortalities are mainly restricted to young animals.

The main clinical signs are anorexia, weakness, sometimes a haemorrhagic diarrhoea, and epistaxis particularly towards the end of an outbreak. A focal necrotic or ulcerative stomatitis may be seen in some animals. Some may show severe purpuric haemorrhage. Thrombo- and leukocytopaenia is common and mortality may range from 90–100%. The main features at autopsy are large haemorrhages in the skeletal muscles, subcutaneous tissue, mucous membranes and under serous surfaces. Ulceration of the buccal cavity and tongue is present while secondary infection by *Mucor* of the fore-stomach, intestine and liver, is a prominent lesion. An outbreak of dermal toxicity and subsequent general toxicosis following exposure to toxic bedding has been documented. Sheep are weak, anorectic, show a nasal catarrh, epistaxis and a haemorrhagic diarrhoea and die within 1–2 days.The fleece may be easily pulled out and numerous haemorrhages may occur in the skin. Pathologically and bacteriologically the appearance at autopsy resembles septicaemic pasteurellosis. Haemorrhages are present in the subcutaneous connective tissue, skeletal muscles, and the serous and mucous membranes. *Pasteurella haemolytica* may be isolated from the organs of some animals. This is considered to be a secondary infection as a consequence of the immunosuppression caused by the fungal toxin. In swine the disease is caused by either contaminated food or bedding resulting in three main forms of the disease, i.e. a dermal form in suckling pigs and sows manifesting as a scabious or necrotic dermatitis affecting contact areas, a general toxicosis resembling that of the horse, and abortions. Inhalation of toxic material in humans results in nasal catarrh, epistaxis, conjunctivitis and inflammation of the throat[2 13].

Although massive outbreaks of strachybotryotoxicosis have occurred in the U.S.S.R. and eastern Europe, and even though the causative fungus is a cosmopolitan saprophyte[9 14], only isolated reports are available of the occurrence of the disease anywhere else in the world[15 18 24]. This may be due to the existence of toxic and nontoxic strains of the fungus[9 11 15 21], but highly toxic strains have also been isolated in countries where no authenticated field outbreaks have been recorded, e.g. the United States of America[6 11 26]. The explanation for the almost complete absence of recorded cases of sta-

Table 1: FEEDING REGIMES AND MORTALITY FIGURES OF SHEEP EXPOSED TO *STACHYBOTRYS CHARTARUM* INFECTED CUBES

Sheep (sex and number)	Feeding regimes	Cubes fed		Sheep died		Number dead
		From	To	From	to	
Stud rams 5	No grazing. Lucerne cubes *ad lib.* Crushed oats twice daily. Lick[4].	15.1.77	24.6.77	–	–	–
Rams 138[1]	Ample barley grazing (pasture); limited straw cubes; Rumevite[5] blocks.	15.12.76	10.1.77	–	–	–
	Barley grazing (restricted); straw cubes *ad lib.*; Rumevite blocks.	10.1.77	15.2.77	–	–	–
	'Litjies grass' grazing (ample); limited straw cubes.	15.2.77	15.4.77	25.2.77	20.3.77	8
Rams 39	Kraaled; lucerne cubes *ad lib.*; limited amount of crushed coats.	15.4.77	24.6.77	18.6.77	22.7.77	7
Rams 91[2]	Restricted grazing; straw cubes *ad lib.*	15.4.77	10.5.77	–	–	–
Rams 26	Kraaled; lucerne cubes *ad lib.*; limited amount of crushed oats.	10.5.77	24.6.77	28.6.77	14.7.77	3
Rams 65	Restricted grazing; straw cubes *ad lib.*; limited amount of crushed oats.	10.5.77	24.6.77	–	–	–
Breeding ewes (registered) 210	Kraaled; straw cubes *ad lib.*; until parturition, then lucerne cubes *ad lib.*	10.3.77	22.5.77	15.4.77	9.6.77	70
Grade ewes 115	Kraaled; straw cubes *ad lib.*	10.3.77	10.4.77	–	–	–
	Veld grazing *(Cynodon dactylon)*; limited straw cubes.	10.4.77	15.5.77	10.4.77	22.4.77	21
Young ewes 100[3]	Grazing; limited straw cubes	22.4.77	22.6.77	–	–	–
Young ewes 60	Kraaled; lucerne cubes *ad lib.*	12.6.77	24.6.77	–	–	–

1. Group of rams subdivided into 2 groups of 39 and 91, respectively, on 15.4.77.
2. Group of rams subdivided into 2 goups of 26 and 65, respectively, on 10.5.77.
3. Group of 60 of these ewes kraaled on 12.6.77.
4. 60% Salt, 30% bone meal, 5% sulpher, 5% molasses.
5. Rumevite Blocks. National Chemical Products, P.O. Box 286, Bedfordview 2008.

chybotryotoxicosis in other parts of the world, should perhaps be sought in the incorrect diagnosis of field outbreaks and/or the failure to isolate the slow-growing *S. atra* during routine mycological examinations of suspect fodders[9 15 16].

In this paper a field outbreak of a haemorrhagic syndrome in sheep in South Africa is described and some preliminary evidence of the involvement of *Stachybotrys* mycotoxin(s) in the aetiology is presented.

CASE REPORT

History

An outbreak of disease in adult sheep and neonatal lambs characterised by a varying clinical manifestation and a very high mortality rate occurred in a South African Mutton Merino stud in the south-western Cape Province. This is a winter rainfall area in which dietary supplementation during the summer is mandatory.

Mortalities occurred over a period of 6 months during which time sheep cubes prepared on the farm were fed as a supplement to green barley grazing, to grazing on stubble or fallow lands, together with varying amounts of crushed oats, or as the sole diet (Table 1 and Fig. 1.). Mortalities commenced at least one month after continual exposure to sheep cubes and persisted for approximately three weeks to one month after removal of the cubes from the diet. The highest percentage mortality, 33,3 % and 18,2 % occurred in the registered and grade ewes respectively. They were kraaled and fed exclusively on cubes for a period of up

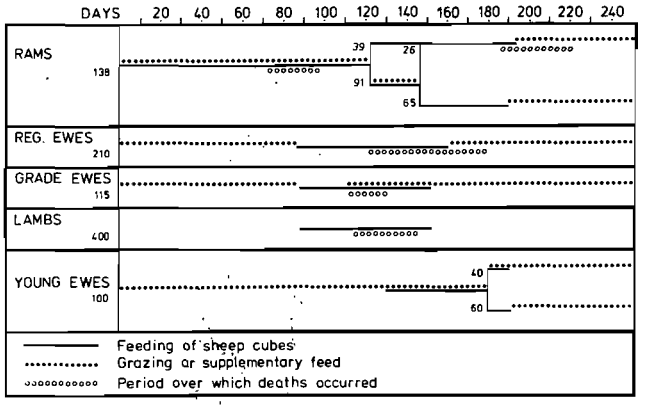

Fig. 1. Relationship between feeding regimes and mortalities due to *Stachybotrys chartarum* infested sheep cubes.

to three months. Addition of crushed oats, Rumevite blocks*, barley or veld grazing resulted in a marked reduction in mortality (of rams), or even prevented the occurrence of the syndrome as in the case of the stud rams. Out of the flock of 568 sheep, 109 died.

Clinical Findings

Adult Sheep.

According to the time elapsed after commencement of cube feeding or supplementation and the onset of clinical signs, the syndrome may be categorised into:

1. Haemorrhagic septicaemic cases (late February –

*Rumevite Blocks, National Chemical Products, P.O. Box 286 Bedfordview 2008.

late May). Some animals died without noticeable clinical signs whereas most were obviously ill, had an elevated body temperature (41–42°C), were dyspnoeic, listless and anorexic. Epistaxis of varying severity was noticed in about 60 % of cases while an intermittent haemorrhagic diarrhoea was often the earliest sign. Haemorrhage from the tips of the ears was rarely noticed.

2. Anaemic cases (late May – August). Similar clinical signs to those observed during the previous stage were observed initially. However, anaemia became progressively severe and constituted the main clinical sign during the latter stages of the outbreak. During this time haematuria was noticed in one animal while another showed persistent haemorrhage from the ear for two days following tattooing. Haemorrhage in this case could only be stopped by cauterization.

The rams, in general, were less obviously ill than the ewes. However, all animals that showed clinical signs, died despite intensive antibiotic treatment and various prophylactic immunisations. Only those animals that received a blood transfusion (markedly anaemic cases) in addition to broad-spectrum antibiotics, responded well. Of these, 15 of 17 recovered.

Lambs.

Lambing lasted from late March to middle May. During this period the ewes were in good condition although udder development was not as good as expected. Parturition progressed uneventfully and the lambs born during the first 14 days of the lambing season were unaffected. During the following 14 days most of the lambs born died without suckling within one to three days after parturition. Mortalities persisted till the end of lambing time. The lambs that died appeared lethargic initially. Thereafter they developed a high body temperature, became more listless and died within 12–24 hours after the appearance of the initial signs of illness. A total of 180 lambs of 400 died during this period. Ewes started dying at the height of the lamb mortalities.

Composition of cubes

Three types of grain (wheat, oats and barley) grown on the affected farm were harvested during November 1976. The straw was left lying on the ground and then baled. The bales were also left lying in the field for several days before they could be stacked. The barley was harvested first and the bales of barley straw (*c.* 600) were placed at the bottom of the stack, followed by *c.* 2200 bales of oats straw and *c.* 2200 bales of wheat straw at the top of the stack. The bales of straw from this stack were used to process the cubes during December 1976 and January 1977 and were also used as bedding in the sheep pens. The rainfall during this entire period of harvesting, baling, stacking and pellet-making was abnormally high (Table 2) and the straw became wet and visibly mouldy.

Bales of either wheat, oats or barley straw, or any combination of the three were used at random from the stack for the processing of two types of sheep cubes, i.e. straw and lucerne cubes. Bales considered to be excessively mouldy were discarded.The composition of the so-called "straw cubes" was as follows:

Table 2: TOTAL MONTHLY RAINFALL FIGURES FOR THE AFFECTED FARM

Month	Total monthly rainfall (mm) Mean[1]	1976/77
November	7,3	70,5
December	9,4	31,2
January	3,2	15,5

1. Mean monthly rainfall for the 8 years preceding the outbreak (November 1968 to January 1976) according to records kept on the farm.

Straw (wheat, oats or barley)	:	50 %
Oats	:	20 %
Barley	:	20 %
Voermol SB 100*	:	10 %

Approximately 200 bales of lucerne hay were purchased and used in the manufacture of the so-called "lucerne cubes". The composition of these cubes was the same as that of the "straw cubes", except that 38 % straw and 12 % lucerne hay were used. The approximate protein content of the straw and lucerne cubes was 6 and 8,6 % respectively.

The feed cubes were spread out to cool after processing and were then placed in jute bags and stored indoors until used. This method of pelleting had been in use on the farm for at least 14 years and no problems had previously been experienced. The cubes were fed from mid-December 1976 until the end of June 1977 according to the regimes set out in Table 1.

Vaccination and treatment

During the course of the outbreak some or all of the animals were vaccinated against enterotoxaemia (*Clostridium welchii* type D), blue tongue, lamb dysentery, *Clostridium septicum*, Rift Valley Fever, Wesselsbron disease and pasteurellosis.

In the case of vaccination against pasteurellosis, the standard Onderstepoort vaccine as well as an autogenous *P. haemolytica* vaccine was administered. None of the vaccinations, including the pasteurella vaccines had any noticeable effect on the course of the disease. Antibiotics employed on the strength of antibiograms of the specific organisms only postponed death for a few days at best. Blood transfusion additional to broad-spectrum antibiotics, was the only effective treatment in the anaemic cases.

LABORATORY EXAMINATIONS

Haematology

The haemoglobin concentration, haematocrit and total white blood cell count were assessed in blood collected from clinically affected rams during the last stages of the outbreak one week after withdrawal of the sheep cubes. In all the cases examined, there was a marked depression in the values of all the parameters (Table 3). A single blood transfusion in addition to the administration of broad-spectrum antibiotics resulted in a marked improvement of the haematocrit within three

*Voermol SB 100. Tongaat Milling (Maidstone) Ltd., P.O. Box 13, Maidstone 4380.

Table 3: BLOOD HAEMOGLOBIN CONCENTRATION, HAEMATOCRIT AND WHITE BLOOD CELL COUNT OF SHEEP PRIOR TO AND FOLLOWING BLOOD TRANSFUSION

Day after treatment	Haemoglobin concentration (g/dl) Sheep no.					Haematocrit (%) Sheep no.					White blood cell count (/mm³) Sheep no.				
	6122	6032	6168	6177	6183	6122	6032	6168	6177	6183	6122	6032	6168	6177	6183
0						15	9,5	19	35	13	7160	11800	2280	8600	1040
3	7,8	5,9	7,4	12,0	7,5	25	17	23	40	23					
6	9,8	7,2	7,0	12,3	7,3	31	21	20,5	40,5	20	4840	C	2400	7360	2400
10	8,8	D	8,9	10,2	7,6	27,5	D	28	34	24					

D = Died C = Clotted

days. This improvement was maintained, remained static or regressed again after six and ten days, respectively. No improvement was seen in the white cell count. However, this treatment proved to be life-saving.

Gross pathological findings

The gross pathological features are summarised in Table 4. According to the autopsy features the cases could also be arbitrarily divided into haemorrhagic-septicaemic and anaemic categories analogous to the classification based on the salient clinical signs. In the early cases there was a marked pulmonary oedema and congestion in all cases whereas there was a variation in degree and occurrence of pneumorrhagia, subserosal haemorrhages, haemorrhages in the tracheal and bronchial mucosae, on the gastro-intestinal tract, subcutaneous and subpleural and subperitoneal haemorrhages. Subpleural haemorrhage in the lungs ranged in size from petechiae to suggillations while those elsewhere ranged from petechiae to ecchymoses. In later cases haemorrhage in the tracheal mucosa appeared to be more constant. Furthermore, haemorrhage in the gastro-intestinal mucosa, a catarrhal enteritis, enterorrhagia and epistaxis were observed. The anaemic cases manifested the entire range of changes described. Varying degrees of anaemia were additionally noticed. The most severely anaemic animals presented less extensive or no haemorrhages while liver degeneration, pneumonitis, nephrosis and renal pigmentation were observed in some of these cases. A single focus of a focal necrotic pharyngitis was observed in one animal. This lesion was found at the base of the tongue in the lateral aspect of the pharynx, was *c.* three cm in diameter, well circumscribed with a hyperaemic border, depressed below the surface and covered by a yellowish pseudomembrane.

Table 4: GROSS PATHOLOGICAL FEATURES OF ADULT SHEEP AUTOPSIED DURING THE COURSE OF THE OUTBREAK

	Clinical categories														
	Haemorrhagic-septicaemic									Anaemic					
Gross lesion	Case no.									Case no.					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	20
Pulmonary oedema	4	4	3	2	4	4	4	3	3	2	2	2	4	1	4
Pulmonary congestion	1	1	3	1	1	1	1	2	3	2	2	1	3	1	4
Pulmonary haemorrhages	0	4	2	4	4	4	4	0	3	2	2	1	0	1	0
Subserosal haemorrhages on gastro-intestinal tract	0	4	2	4	4	4	4	0	3	2	2	0	0	2	0
Subcutaneous haemorrhages	4	0	0	3	2	2	2	0	4	4	4	0	4	4	0
Subpleural and subperitoneal haemorrhages	4	1	4	4	4	4	4	0	3	1	1	0	1	2	1
Tracheal and bronchial mucosal haemorrhages	0	2	0	2	2	2	0	0	1	1	1	0	3	1	0
Gastro-intestinal mucosal haemorrhages	0	0	2	0	3	2	3	0	0	0	0	0	0	2	0
Catarrhal enteritis	0	0	3	1	2	0	2	0	0	2	2	0	3	0	2
Enterorrhagia	0	0	0	2	4	0	4	0	0	0	1	0	0	2	0
Epistaxis	0	0	0	3	0	0	4	0	3	0	0	0	4	0	0
Anaemia	0	0	0	0	0	0	0	4	2	0	0	3	0	1	3
Liver degeneration	0	0	0	0	0	0	3	3	0	0	0	0	0	0	2
Pneumonitis	0	0	0	0	0	0	0	3	0	0	0	2	1	3	0
Focal necrotic pharyngitis	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Renal pigmentation (orange-brown)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
Nephrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3

1–4 = relative severity of lesions 0 = absent

At autopsy various organ specimens were collected aseptically for bacterial isolation, the results of which are presented in Table 5. The most remarkable finding was the presence of *Pasteurella haemolytica* in organ specimens from six of the animals.

No remarkable pathological features were noticed in any of the lambs examined. *Eschericia coli* was isolated from various organ specimens from three of the four neonatal lambs while *Staphylococcus* sp. was isolated from the two-week-old lamb (Table 5).

Histopathology

Tissue and organ specimens from 11 of the 20 autopsies were preserved in 10 % formalin and processed in the conventional way for light microscopic examination. The selection of organs in most cases was by no means complete, and the variety of organs taken expanded as the disease problem progressed in the flock.

The lymphoid tissue throughout the body was affected and showed varying degrees of depression and necrosis of varying extent. The spleen was most severely affected, the lymph nodes to a varying degree and the submucosal lymphoid tissue least. In some only primary nodules were present whereas in others, particularly during the later stages of the outbreak, the germinal centres were depleted of lymphocytes while

Table 5: BACTERIA ISOLATED FROM AUTOPSY SPECIMENS FROM ADULT SHEEP AND LAMBS

Case No.	Date	Bacteria isolated	Isolated from
Adult sheep Haemorrhagic septicaemic cases			
1	14.3.77	No specimens submitted	Too decomposed
2	17.3.77	*Clostridium septicum*	Spleen, liver, brain, small intestine
3	25.4.77	*Pasteurella multocida*	Spleen, liver lung
4	2.5.77	*Cl. perfringens; Eschericia coli*	
5	4.5.77	*P. haemolytica*	Liver, spleen, brain, lung, lymph node
6	4.5.77	*P. haemolytica*	Liver, spleen, lung
7	5.5.77	*P. haemolytica*	Liver, spleen, lung
Anaemic cases			
8	30.5.77	*P. haemolytica*	Lung, mammary gland
9	30.5.77	Negative	
10	30.5.77	*P. haemolytica*	Lung, spleen, brain
11	30.5.77	*P. haemolytica*	Lung, brain, spleen
12	20.6.77	Mixed culture	Treated with antibiotics
13	23.6.77	Negative	Not treated
14	28.6.77	Negative	Treated with antibiotics
20	11.7.77	No specimens submitted	Too decomposed
Lambs			
15	15.4.77	*Escherichia coli*	Spleen, umbilicus
16	4.5.77	*Klebsiella* sp.; *Enterobacter* sp.	
17	4.5.77	*E. coli; Klebsiella* sp.	Spleen, small intestine
18	4.5.77	*E. coli*	Liver, spleen, brain, lung
19	30.5.77	*Staphylococcus* sp.	Two-week-old lamb

necrotic cellular debris and lipofuscin pigment were present in the remaining framework of reticular cells. Furthermore there was a decrease, sometimes very marked, in the number of lymphocytes in the marginal zones and cords of Bilroth in the spleen. A marked reduction in lymphocytes in the medullary cords of the lymph nodes and in the submucosal lymphoid aggregates, was evident.

The bone marrow, obtained from two cases three and 14 days after removal from the sheep cubes, respectively, manifested a marked supression of the granulocytic series while a few areas of focal necrosis were evident in the former.

The reaction to a marked parasitic pneumonia *(Muellerius capillaris)* in two cases reflected an impeded immune and inflammatory response. Lymphocytes and granulocytes were conspicious by their absence while alveolar macrophages were the predominant cell type. In eight of the 11 cases there was bacterial embolism without any accompanying leucocyte reaction.

Haemorrhage was observed in the lungs, some of the lymph nodes, oral and respiratory mucous membranes and kidneys. Most of the cases showed moderate accumulations of haemosiderin in the liver, spleen and kidneys, the most extensive accumulation being noticed in the proximal convoluted tubules of the animals in the anaemic stage of the outbreak.

Acute, nonspecific toxic or hypoxic degenerative changes were observed in the liver and kidney of most cases. In three, focal, coagulative necrosis involving the glomeruli and tubuli was noticed in the kidneys. In these cases there was haemorrhage into the tubular lumina in association with occasional bacterial colonies and few neutrophils. Focal, periportal, coagulative necrosis was present in one case.

Chronic and probably incidental lesions of mild interstitial nephritis, bile duct proliferation in the liver and a parasitic pneumonia occurred in most of the cases.

Mycotoxicological investigation

Mycology.

Samples of visibly mouldy wheat, oats and barley straw were collected during June 1977 from bales stored indoors in a stack on a farm where the outbreak had occurred. Microscopic examination of a very conspicuous sooty black fungus on the wheat straw, revealed it to be a species of *Stachybotrys.* This fungus was observed on the sample of wheat straw and not on the oats or barley straw, although other unidentified black fungi were present on these straw samples. Subsamples of wheat, oats and barley straw were ground to fine meal in a Wiley mill, 25 g of each meal suspended in 250 ml of distilled water, shaken on a mechanical shaker for 5 minutes, and the numbers of *Stachybotrys* conidia in each sample counted in a haemacytometer as described for *Pithomyces chartarum* (Berk. & Curt.) M.B. Ellis by Di Menna & Bailey[4]. According to this method, the number of *Stachybotrys* conidia present in the ground wheat straw was 8,5 million/g, while none were present in either the ground oats or barley straw. The samples examined were, however, not representative of the entire stack and subsequent examination of additional straw samples from the farm revealed that the *Stachybotrys* sp. was also present on the barley and probably on the oats straw as well. A great deal of variation was also observed in the amount of *Stachybotrys* contamination present in different bales of wheat straw. Some bales were sooty black with conidia, some had localised areas of infestation, while other were not visibly invaded.

The *Strachybotrys* sp. was isolated from the wheat straw as follows: A small piece of straw colonized by

this fungus was shaken in sterile water in a test tube, a dilution series of the spore suspension prepared in sterile water and planted on water agar, the conidia allowed to germinate overnight, and single germinating conidia isolated under a stereomicroscope and transferred to slants of either potato dextrose or oat meal agar. Stock cultures of single conidial isolates were incubated at 20°C under near ultra-voilet light radiation until profuse sporulation occurred and were then stored at 25°C.

Sporulating cultures of the *Stachybotrys* sp. isolated from the wheat straw were identified according to the key of Jong & Davis[14] as *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes. This fungus is known in the literature under a variety of synonyms, notably *S. atra* Corda and *S. alternans* Bon.[14]. A detailed account of the morphology of the present isolates of *S. chartarum* will be published elsewhere.

Because of the observed variability in the level of *Stachybotrys* infestation of the wheat straw, an amount of heavily infested blackened straw was hand-selected from several bales for use in further chemical and toxicological investigations. This straw was also ground in a Wiley mill and yielded a sooty black meal which contained 36,5 million *S. chartarum* conidia/g. A bale of good quality commercial wheat straw was used as a control. Meal prepared from this control straw contained no *S. chartarum* conidia. In order to obtain a representative sample of straw cubes, several bags of cubes were mixed and the resulting mixed sample of cubes used in subsequent chemical and toxicological investigations.

Bulk cultures of *S. chartarum* for dosing to experimental animals and chemical analyses were prepared as follows: Conidial suspensions from sporulating stock cultures were prepared in sterile water and used to inoculate autoclaved, yellow maize kernels (400 g kernels in 400 ml water, autoclaved at 103 KPa for one hour on each of two consecutive days) in two litre glass jars. The cultures were incubated at 20°C for 4 weeks[6 15 16 17 20]. The content of the jars were then harvested, dried overnight at 45°C, ground to a fine powder in a Wiley mill and stored at 5°C until used. Initially culture material of nine single conidial isolates of *S. chartarum* was prepared in this way.

Chemistry

Straw cubes, wheat straw and *S. chartarum* culture material were extracted according to the procedure for obtaining stachybotryotoxin A from *Strachybotrys* cultures[20]. Ground wheat straw (1,2 kg) and straw cubes (1,5 kg) were extracted with diethyl ether in a Soxhlet apparatus for 18 hours. The extracts were filtered, concentrated under reduced pressure, and subsequently precipitated in petroleum ether. The extracts on the plates contained fractions corresponding to those in a sample of stachybotryotoxin A[20], obtained for comparison from Dr M. Palyusik, Veterinary Medical Research Institute, Budapest, Hungary.

Diethyl ether extracts were prepared in a similar way of the mixed straw cubes (2,5 kg), hand-selected *S. chartarum* infested wheat straw (1,4 kg), control wheat straw (1,6 kg) and ground maize culture material of *S. chartarum* isolate MRC 1000 (200 g) and a similar range of components found on thin layer chromatography.

Toxicity tests.

Ground straw cubes and wheat straw fed *ad libitum* to groups of four one-day-old Pekin ducklings and weanling white Wistar-derived rats caused the death of 4/4 ducklings and rats within six and nine days, respectively. No mortality was observed within 14 days in rats fed a diet of ground straw cubes or wheat straw mixed with commerical rat mash (1:1, m.m).

Crude diethyl ether extracts and petroleum ether precipitates of the wheat straw and cubes were suspended in propylene glycol and 0,05 ml of each injected intradermally on the clipped back of a white Wistar rat[20]. The control rat was injected with propylene glycol only. The extracts as well as precipitates caused a positive oedematous to necrotic skin reaction (Table 6). The extract of the wheat straw caused a more severe reaction than that of the cubes, and in both cases the reaction to the crude extract was more severe than to the precipitate (Table 6).

Table 6: SKIN REACTION OF RATS TO INTRADERMAL INJECTION OF EXTRACTS OF STRAW AND STRAW CUBES[1]

Extract no.	Source	Skin reaction[2]
Control	Propylene glycol	0
1	Wheat straw, diethyl ether crude extract	4
2	Straw cubes, diethyl ether crude extract	3
3	Wheat straw, petroleum ether precipitate of No. 1.	2
4	Straw cubes, petroleum ether precipitate of No. 2	1

1. Extract injected into clipped skin; each extract applied to a different rat.
2. Graded according to an arbitrary scale from 0 to 4 based on the severity of the oedema, hyperaemia and necrosis present after 3 days.

Crude extracts of the mixed cubes, hand-selected *S. chartarum*-infested straw and control straw were suspended in olive oil (0,5 ml concentrated extract in 1,5 ml olive oil) and injected intradermally in rats.Extracts of the cubes and infested straw caused severe necrotic skin reactions, while the control wheat extracts caused no visible reaction (Table 7).

Table 7: SKIN REACTION OF RATS TO INTRADERMAL INJECTIONS OF EXTRACTS OF MIXED STRAW CUBES HAND-SELECTED *STACHYBOTRYS*-INFESTED WHEAT STRAW AND CONTROL WHEAT STRAW[1]

Extract No.	Source	Skin reaction[2]
1	Control: Non-infested wheat straw	0
2	Hand-selected *Stachybotrys*-infested wheat straw	2
3	Mixed straw cubes	4

1. Extract injected into clipped skin; each extract on a different rat
2. Graded according to an arbitrary scale from 0 to 4 based on the severity of the oedema, hyperaemia and necrosis present after 3 days.

Mouldy maize meals prepared from cultures of nine single conidial isolates of *S. chartarum* were mixed with commercial chicken mash (1:1, m/m) and fed *ab lib.* to groups of four one-day old Pekin ducklings for 14 days

Table 8: TOXICITY TO DUCKLINGS OF *STACHYBOTRYS CHARTARUM* CULTURE MATERIAL[1]

Isolate no.	Mean mass gain (g)	Mean feed intake (g)	Post Mortem
MRC 997	144,5	400	No abnormalities
MRC 999	178,5	550	One with linear ulceration in pharynx, ca. 0,5 cm long
MRC 1000	140,5	550	One with linear ulceration in pharynx, ca. 0,5 cm long
MRC 1001	127,75	550	No abnormalities
MRC 1002	211,25	550	No abnormalities
MRC 1003	181,5	550	No abnormalities
MRC 1004	144,5	529	No abnormalities
MRC 1005	152,0	400	One with single large ulceration, ca. 0,75 cm in diameter
MRC 1006	202,0	550	No abnormalities
Control	269,75	827	No abnormalities

1. *S. chartarum* culture material mixed with commercial chicken mash (1:1, m/m) fed to groups of 4 1-day old Pekin ducklings for 14 days. The control group received uninoculated maize meal instead of culture material.

(Table 8). None of the *S. chartarum* strains caused mortalities, but all caused reductions in mass gain and feed intake compared to control ducklings. One duckling in each of three groups that received culture material had visible ulcerations in the pharynx. On the basis of the presence of a large ulcer in one duckling fed culture material of isolate MRC 1005, and because of the marked reduction in mass gain and feed intake of the ducklings in this group, isolate MRC 1005 was selected for further study.

The results of further toxicity trials with the straw cubes and *S. chartarum* culture material will be reported elsewhere.

DISCUSSION

This is the first report of an authenticated outbreak in sheep of disease due to the ingestion of *S. chartarum*-infested feed in any country outside of the U.S.S.R. and certain Eastern European countries.

The history, major clinical signs and autopsy features resemble those described for sheep[9 13], and the presence of trichothecene-like mycotoxins in the sheep cubes could be demonstrated biologically and by thin layer chromatography. The cause of death in a large number of cases, but excluding the lambs, appeared to be due to a septicaemic *P. haemolytica* infection. This increased susceptibility to infection has been attributed to an impaired immunological response as a consequence of the intoxication[2 3]. This type of intoxication and the consequent immunological impairment may very well constitute one of the unknown predisposing factors necessary to precipitate *P. haemolytica* biotype T septicaemias which are notoriously difficult to induce experimentally even with massive numbers of infective organisms[12].

Several unusual features appeared to be necessary for this condition to manifest itself locally. The unseasonal rain and accompanying humid and hot conditions no doubt contributed to the massive proliferation of the fungus in the different grain straws. The fact that the material was ground and incorporated into sheep cubes forced the animals to ingest material over a prolonged period which they could otherwise have avoided. The local observation that sheep will ingest unspoilt hay in preference to mouldy hay from a stack, is in sharp contrast to the observation of Danko[2] who reported a selective ingestion by sheep of *Stachybotrys*-infested hay which he ascribed to an improved taste of the affected material. It should however be pointed out that all the black fungi observed on straw can by no means assumed to be *S. chartarum*. Final identification is only possible through mycological examination and both these observations appear to be of little consequence in this specific outbreak. The rather selective growth characteristics of this fungus was also well demonstrated by the fact that only parts of particular bales were involved while many of the bales were not affected at all. In the affected bales there was a sharp line of demarcation between fungus-infested and unspoilt hay. These variations may result in a varying toxin content of particular cubes and batches of cubes.

A further important factor is the observation that, with a few exceptions, only those animals exclusively fed contaminated cubes over an uninterrupted period of at least 30 days, manifested clinical signs and died. Whether the additional nutrients protected the animals or whether it can simply be ascribed to an effective dilution of the amount of toxin present, remain to be resolved. In the final analysis, both these factors may prove to be important. These factors may furthermore also be influenced by the varying toxicity as discussed above.

A few observations reported here differ from the descriptions elsewhere in the literature[9 13]. Very few lesions were noticed in the oral cavity of these sheep. In fact, in only one case was a single ulcer found in the pharynx while none of the lesions previously described to occur on the lips and in the oral cavity, were present. Another prominent feature of this outbreak not emphasized previously, is the very marked anaemia that was observed in the later stages of the outbreak. This appeared to be a contributory cause of death in the animals which did not succumb to a *Pasteurella* infection. The importance of the anaemia in this respect is further emphasized by the fact that blood transfusion was the only life-saving treatment. It is uncertain which component or combination of components of the whole sheep's blood transfusion was life saving, i.e. the red blood cells, white blood cells or the passive transfer of antibodies. That the bone marrow depression induced by these toxins is reversible, albeit slowly, is supported by the observation that mortalities ceased only 15–20 days after the cubes were removed from the diet or after introduction of supplementary feeding. Even after a blood transfusion, recovery was slow although there was an apparent marked clinical improvement.

The concommitant outbreak of early postnatal mortalities in lambs was in all probability due to a septicaemic *E. coli* infection. It is conceivable that this may also have been precipitated by the toxins due possibly to direct toxic effects on the foetus and/or to low antibody levels in the colostrum although extensive mortalities due to this organism are by no means uncommon.

This condition poses a diagnostic problem. It is characterised by varying clinical and pathological manifestations extending over a long period of time. These appear to be influenced not only by varying concentrations of the toxin in the diet, but probably also by the

complex nature of the Stachybotryotoxins produced by different strains of the fungus[13]. Even though a presumptive diagnosis may be made on the history, clinical signs and pathological changes, a final diagnosis may only be made following the demonstration of the *Stachybotrys* toxin(s) in any or a number of the dietary components.

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BOOK REVIEW

BOEKRESENSIE

BOVINE MASTITIS

W.H. GIESECKE
Technical Communication No. 51, ISBN0621 04720 1.
Department of Agricultural Technical Services, Pretoria 1979, pp III 38, Figs 27 Tabs 15, No charge.

This Technical Communication is designed to familiarize persons who are particularly interested in the subject of mastitis and its effect on production and quality of milk with some aspects affecting the efficient producion of wholesome milk. To that end it provides general informaion on milk as a food as well as certain physiological and morphological features of the udder which are of major importance to milk production, quality and the maintenance of udder health. Relevant details compiled from international data are summarised in the figures, tables and schedules (4).

The Communication, which is available on request from the Director, Division of Agricultural Information, Private Bag X144, 0001 Pretoria, will find its greatest value when used by the veterinarian to illustrate and emphasize certain matters during his discussions with milk producers who are keen to know and understand more about the udder and its function in health and disease.

L.W. v.d. H

THE USE OF A SHORT AND A LONG ACTING OXYTETRACYCLINE FOR THE TREATMENT OF *ANAPLASMA MARGINALE* IN SPLENECTOMIZED CALVES

C.G. STEWART*, A. IMMELMAN*, P. GRIMBEEK** AND DRICKY GRIB*

ABSTRACT: Stewart C.G.; Immelman A.; Grimbeek P.; Grib Dricky. **The comparative efficiency of a short and a long acting Oxytetracycline for the treatment of *Anaplasma marginale* in splenectomized calves.** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 83 (En) Fac. Veterinary Science, Univ. of Pretoria, Box 12580, 0110, Onderstepoort, Rep. of South Africa.

Twenty splenectomized Afrikander/Simmentaler cross animals were used. Eight of these were naturally infected with *Anaplasma marginale* while 12 were infected artificially. When the packed cell volume was reduced to between 24 % and 20 % during the post splenectomy relapses or primary infections, six animals were treated with a single injection of 20 mg/kg of long acting oxytetracycline (LA) and seven animals were treated on two successive days with 10 mg/kg of short acting oxytetracycline (SA). The remaining seven animals served as untreated controls.

Both drugs were effective in controlling *A. marginale* reactions. No deaths occurred in the group of animals treated with oxytetracycline LA and they showed a longer relapse interval and higher blood levels of oxytetracycline compared to those treated with oxytetracycline SA. A single death occurred in the latter group. Four deaths occurred in the control group which also had the shortest relapse interval.

INTRODUCTION

A number of drugs have been used in the treatment of acute anaplasmosis. Specific action against *Anaplasma marginale* has been shown for the tetracycline group of antibiotics, chlortetracycline[4 8], oxytetracycline[8], tetracycline[2], roli-tetracycline[10] and doxycycline[6]. Other drugs which have been shown to have an inhibitory action on the development of *Anaplasma marginale* are dithiosemicarbazone[1], imidocarb[9] and amicarbalide[3].

Chlortetracycline used at 11 mg/kg intravenously as a single dose in 32 splenectomized calves at various stages of parasitaemia prevented any further increase in parasitaemia[8]. Oxytetracycline at 5,5 mg/kg gave similar results[8]. Roli-tetracycline given at 4, 3 and 2 mg/kg on three successive days was effective in 15 field cases of anaplasmosis whereas treatment was unsuccessful in two cases[10].

Recently a long acting oxytetracycline† (LA) containing 200 mg oxytetracycline/ml became available for the treatment of anaplasmosis[6 7]. A single treatment with this drug gave similar results in both splenectomized[6] and non splenectomized[7] animals as compared with multiple treatments of a standard oxytetracycline†† preparation containing 50 mg/ml.

Splenectomized calves have been used as test animals for therapeutic trials against *Anaplasma marginale* infection[8]. Adult carrier animals will undergo a relapse following splenectomy and calves which normally only develop mild disease become susceptible to acute anaplasmosis when splenectomized.

At present the only drugs registered in South Africa for use by farmers for the treatment of anaplasmosis are the tetracyclines on their own or in combination with other compounds. The accepted minimum dose is 8 mg/kg (Registrar of Veterinary Medicine's personal communication), however the usual dose used under field conditions is 10 mg/kg.

This trial was carried out to compare the relative activity of a shortacting oxytetracycline††† (SA) with that of the oxytetracycline LA against *Anaplasma marginale* in splenectomized cattle.

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**P.O. Box 8692, Johannesburg 2000
† Terramycin Injectable Long acting – Pfizer Laboratories
†† Liquamycin – Pfizer Laboratories
††† Terramycin 100 – Pfizer Laboratories

MATERIAL AND METHODS

Twenty-one Afrikander/Simmentaler cross animals which were negative to the standard complement fixation (CF) test for anaplasmosis were obtained from South West Africa. These animals were approximately 10 months of age with a mass of ± 200 kg. All animals were splenectomized after arrival and blood smears examined three times weekly for 40 days after which they were entered into the trial. They were sprayed weekly with an effective ixodicide throughout the trial.

For 60 days following treatment blood samples were collected from the jugular vein three times weekly in venoject tubes using EDTA as anticoagulant. Smears prepared from this blood were stained with 10 % Giemsa and the parasitaemia estimated by counting the number of parasites in 15 oil-immersion ($X10^3$) fields. The packed cell volume of the EDTA collected blood was measured in microcapillary tubes using a microcentrifuge*.

Anaplasma marginale was obtained from an animal which relapsed to anaplasmosis after splenectomy (this animal was subsequently excluded from the trial). This blood was frozen in 1 ml ampoules in liquid nitrogen at a parasitaemia of 20 % using DMSO as a cryo protectant. A further eight animals proved to be naturally infected while the remaining 12 animals did not relapse to anaplasmosis within 40 days of splenectomy and were inoculated intravenously with 1 ml of frozen blood. Animals were treated when the packed cell volume was reduced to between 24 % and 20 % irrespective of the level of parasitaemia.

The splenectomized cattle were divided into three groups:

Group I received no treatment.
Group II received two treatments of the oxytetracycline SA at 10 mg/kg intramuscularly at an interval of 24 hours.
Group III received a single treatment of the oxytetracycline LA intramuscularly at a dose of 20 mg/kg.

The concentration of oxytetracycline in the blood was assayed spectrofluorometrically using the method of Ibsen *et al.*[5]. Blood samples were collected from all animals in heparin from the jugular vein before each treat-

* Damon IEL Model MB

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ment and one, two, four, six, eight and 24 h after treatment. Thereafter samples were collected twice daily until oxytetracycline could no longer be detected in the blood.

The replase interval was taken as the number of days from treatment to the day of maximum parasitaemia at relapse. Those animals which died from the primary parasitaemia were recorded as having a replapse interval of nought days. Those which did not relapse during the 60 days of the trial were recorded as having a relapse interval of 60 days.

RESULTS

Eight animals relapsed to anaplasmosis after splenectomy; three in group I and II and 2 in group III. These animals presumably became infected between collection of the blood sample for CF testing and splenectomy. They were included in the trial. Three animals died in the control group during the primary reaction while one control and one animal in group II died of anaplasmosis 20 and 40 days respectively after the primary parasitaemia. There were no deaths in group III.

The mean number of days between the primary parasitaemia and the first relapse parasitaemia was 56 days in group III, 38 days in group II and 18 days in the control group. These results are shown in Table 1.

Following the administration of oxytetracycline LA a peak blood level of oxytetracycline of 4,5 mg/ml was reached after six hours, which then steadily declined. The level of 1 mg/ml was reached after 76 hours. The last positive sample was recorded at 102 hours.

Oxytetracycline SA was administered on two successive days. Following the first administration a peak blood level of 2,7 mg oxytetracycline/ml was measured after four hours which fell to 1 mg/ml after 24 hours. The second peak following the second injection was higher than the first and attained 3,2 mg/ml 28 hours after the first injection. At 30 hours the blood level was close to the blood level of the tetracycline LA with a very similar subsequent rate of decline (Fig. 1.).

DISCUSSION

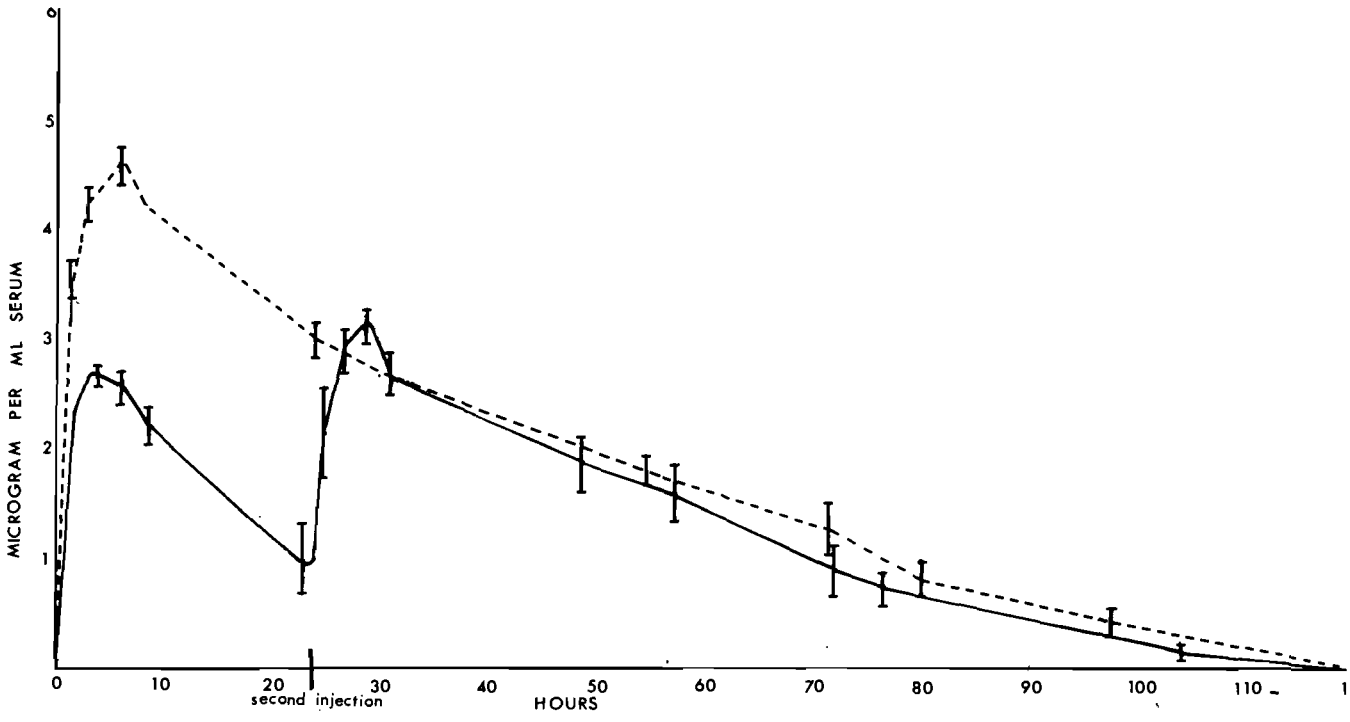
Both oxytetracycline SA and oxytetracyline LA were effective in the treatment of anaplasmosis in splenectomized animals. A single death occurred in the group treated with oxytetracycline SA and none in the tetracycline LA group indicating that a single treatment with this drug may be slightly superior to two treatments with oxytetracycline SA at 24 h intervals.

The significance of the relapse interval as a measure of thereapeutic efficiency is not known but it is thought that it is an indication of the suppressive activity of the drug on *Anaplasma marginale*. A longer relapse interval indicates greater suppression of the parasite. If this is so it supports the view that the single treatment with oxytetracycline LA was slightly superior to a double treatment with oxytetracycline SA. T tests showed a significant difference between these two groups ($P < 0{,}01$). The total amount of drug given in each case was the same.

Kuttler and Simpson[6] found no difference between a single treatment of oxytetracycline LA (20 mg/kg) compared with three treatments with a short acting oxytetracycline (10 mg/kg), given on consecutive days to splenectomized calves. Comparison of these results with the results obtained in the present study indicate that in splenectomized calves a single treatment with oxytetracycline LA is equivalent to three treatments with the short acting oxytetracycline but is possibly superior to two treatments with oxytetracycline SA. The oxytetracycline used by Kuttler and Simpson[6] contained propylene glycol as a vehicle as compared to polyvinnyl pyrrollidone used in the present study. There is no published evidence that this might affect the efficacy of oxytetracycline.

TABLE I

Group I	Natural infection	Deaths	Max parasitaemia	Lowest P.C.V.	Relapse interval	Max level	Tetracycline in blood
10	+	+	40%	6,5	0		
14	+	+	28%	11,5	0		
65	+		25%	16,5	60		
68		+	2%	13,5	0		
13			18%	22	23 days		
199		+ (relapse)	30%	9,7	20		
102			40%	9	25 days		
Mean			26%	13	18,29		
Group II						1st Inoc.	2nd Inoc
61	+	+ (relapse)	30%	17	40 days	2,85	3,35
9	+		12%	24	60	2,75	3,45
69	+		2%	20	25	2,75	3,25
64			10%	19	38	2,7	3,35
126			4%	21	24	2,75	3,05
159			16%	15	32	1,75	3,20
100			18%	10	44	2,75	3,25
Mean			13%	18	37,57	2,76	3,27
Group III							
3	+		40%	14	42	4,25	
125	+		27%	19	60+	4,55	
54	+		34%	13	52	4,85	
60			44%	11,5	60	4,45	
62			14%	20	60+	5,15	
194			35%	14	60+	4,65	
Mean			32%	15	55,66	4,91	



------ Oxytetracycline LA. Oxytetracycline SA. I Standard deviation.

Fig. 1. Mean blood levels of 2 oxytetracycline formulations in *A. marginale* infected splenectomized cattle.

Higher levels of oxytetracycline were obtained in the blood after treatment with oxytetracycline LA as compared to levels obtained with oxytetracycline SA. This would be as expected as 20 mg/Kg of the oxytetracycline LA was given as compared with an initial dose of 10 mg/Kg for the oxytetracycline SA. The maximum concentration of oxytetracycline in the blood after treatment with oxytetracycline LA was 4,5 mg/ml after six hours as compared with 2,7 mg/ml after four hours and 3,2 mg/ml after 28 hours following treatment with the oxytetracycline SA. The only time that the oxytetracycline SA gave a higher blood level than the oxytetracycline LA was following the second treatment (26 hours to 30 hours). The difference in blood oxytetracycline level during these four hours was small and reached a maximum of 0,71 mg/ml.

ACKNOWLEDGEMENTS

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REVIEW **OORSIG**

DIE PINEALE KLIER

H.M. TERBLANCHE

ABSTRACT: Terblanche H.M. **Die pineale klier.** 'n Beknopte samevatting van die anatomie, evolusie, biochemie, endokrinologie en fuksies van die pinealer klier. *Journal of the South African Veterinary Association* (1979) **50** No. 2, 87 (Afr/En). Dept. of Genesiology, Faculty of Veterinary Science, Box 12580, Onderstepoort 0110, Rep. of South Africa.

The pineal gland is part and parcel of the epithalamus connected by a short stalk to the roof of the third ventricle. In fish, amphibians and to a lesser extent in reptiles the gland consists of neurosensitive photoreceptive cells and supporting cells but in mammals regression of the photoreceptive cells has occurred. The gland is richly innervated by postsynaptic noradrinergic fibres. A very brief review is given of the connections of the hypothalamus as well as the specific innervation of various hypothalamic areas. The more important connections centre around the sensitivity of the pineal gland to light stimuli and it is thought that these stimuli reach the pineal by way of the retina, inferior accessory optic tract, medial forebrain bundle, rostral tegmentum, superior cervical ganglion and post-synaptic noradrinergic sympathetic fibres.

Evolution of the pineal gland has resulted in the transformation of the primitive photoreceptive cells found in fish and amphibians into photosensitive neurosecretory cells known as pinealocytes.

The most important biochemical characteristic of the pineal gland is the presence of an enzyme (HIOMT) which is directly responsible for the conversion of N-acetyl serotonin into melatonin. This enzyme is only found in the pineal gland (some exceptions are known) thus making the gland unique as far as melanonin synthesis is concerned. Various other biochemical substances are however found in the pineal but they are all involved in the metabolic pathway of tryptophan and its conversion to melatonin and its metabolic end products.

The cellular organisation of the pinealocyte and the role of noradrenalin and cAMP in the synthesis of melatonin as well as other biochemical substances found in the gland are discussed in some detail. Due to the photosensitive properties of the gland, biological rhythms occur in the biochemical substances and activity of the gland. Duirnal and circadian rhythms are related to the exposure of the gland to light and/or dark stimuli.

Darkness will bring about increased levels of amongst others noradrenalin, N-acetyl serotonin and melatonin while light stimuli will have the opposite effect.

Pineal functions are reviewed to some extent and particular attention is paid to the antigonadotrophic functions of the pineal gland including ovarian atrophy, inhibition of LH synthesis and suppression of sexual maturation. These function are also borne out by the results of pinealectomy experiments where stimulation of gonadotrophin synthesis, ovarian hypertrophy, testicular hypertrophy and other positive effects have been observed.

Pineal endocrinology is also reviewed in some detail with particular emphasis on substances such as argenine vasopressin, melatonin, as yet unidentified polypeptide factors, 5 OH tryptophol and 5 methoxytryptophol. The biological effects of melatonin are discussed in greater detail including suppression of reproductive functions, antigonadotrophic effects, delay in the onset of puberty, the anti- LH effects and inhibition of ovulation and pituitary functions.

The influence of hormones on pineal activity is also briefly mentioned. Oestrogens seem to be stimulatory in normal physiological doses but inhibitory in high doses. A specific cytosol receptor for oestrogens has been found to exist in the pineal gland.

It is finally concluded that the pineal gland is primarily photosensitive, that its biochemical activity is stimulated by darkness and inhibited by light stimuli and that its function is primarily anti-gonadotrophic. The exact role of the pineal in domestic animals is however not known. It may play a role in those species where seasonal reproduction occurs but the exact mechanisms still remains to be fully understood.

PINEALE ANATOMIE EN NEUROLOGIE

Die pineale klier maak 'n deel uit van die epitalamus van die brein en lê in die volwasse dier net agter die habenula, ventraal van die knie van die *corpus callosum,* dorsaal van die boonste kollikulus en tussen die twee vry uitbultende lobbe van die talamus[44 45] (Sien Fig. 1). Dit is verbind met die dak van die derde ventrikel deur middel van 'n kort steeltjie[44 45].

In die bees ontstaan die pineale klier in die fetus as twee aparte lobbe, nl. 'n rostrale deel tydens die vyfde en sesde week van dragtigheid, vanuit spongioblaste en 'n kaudale deel tydens die 17e week van dragtigheid vanuit gespesialiseerde sekretoriese epiteel anlage[44].

Die pineale klier van visse en amfibië bestaan uit neurosensoriese fotoreseptiewe selle[44], ondersteunende selle en sensoriese senuweeselle[18]. In reptiele is daar 'n regressie van die fotosensitiewe selle en in voëls is hierdie selle totaal en al afwesig[18]. In die soogdier geld dieselfde toestand en hier vind ons dat die aktiewe pineale sel, die pinealosiet, uit die fotosensoriese selle van die laer vorme ontwikkel het[18 31].

Die pineale klier van die soogdier is ryklik voorsien van outonome senuwees van die postsinaptiese noradrinergiese (simpatiese) stelsel[4 18]. Hierdie vesels het hulle oorsprong in die boonste servikale ganglion en bereik die pineale klier langs een en/of meer van die volgende maniere:

(a) Deur die afskeiding van noradrenalien wat die klier deur die perivaskulêre spasies bereik
(b) Simpatiese vesels wat deur die oppervlaktte van die klier dring tot in die klierweersel
(c) Simpatiese vesels wat in die Nn. conarii tot in die klierweerfselloop[18].

Hierbenewens is daar ook bewyse van cholinergiese vesels (parasimpaties) in die pineale kliere van beeste, skape, varke en primate[18 20 44]. Benewens hierdie senuweevoorsiening is daar geen ander afferente of efferente verbindings tussen die brein en pineale klier nie[18].

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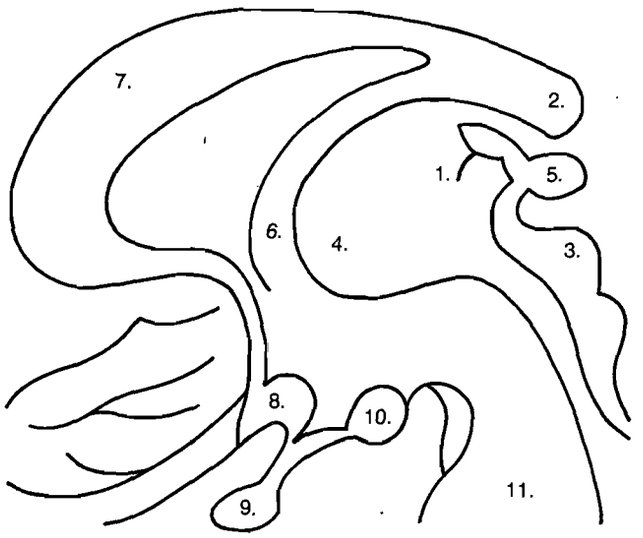


1. Habenula
2. Knie van die *corpus callosum*
3. Superior colliculus
4. Talamus
5. Pineale klier
6. Forniks
7. *Corpus callosum*
8. Optiese kiasma
9. Hipofise
10. Mammilêre liggaam
11. Medulla

Fig. 1. Ligging van die pineale klier in verhouding tot die brein. (Aangepas uit Truex & Carpenter[45])

Aangesien die hipotalamus in noue verband staan met baie van die pineale funksies soos ons later sal sien, is dit volledigheidshalwe nodig om eers vir 'n wyle stil te staan by die neurale konneksies van die hipotalamus en die verskillende bane wat tussen die brein en hipotalamus aangetref word asook 'n uiteensetting van die tipe innervering wat betrokke is by die vrylating van die hipotalamiese hormonale faktore.

Die kerne van die hipotalamus lê meestal mediaal terwyl die veselbane net tot in die laterale gebiede strek[33]. Die stygende en dalende bane in hierdie gebied is bekend as die mediale voorbrein bondel (M F B)[33]. Die afferente konneksies van die hipotalamus loop hoofsaaklik in 'n stygende baan deur die breinstam en in 'n dalende baan deur die M.F.B. vanaf die hipokampus, amygdala en piriforme skors[33]. Die efferente funksies word uitgeoefen deur 'n endokriene effek of deur die MFB in 'n stygende rigting na die amigdala, piriforme skors en septum en in 'n dalende rigting na die outonome kerne[33]. (Sien Fig. 2).

Die sensoriese afferente bane na die hipotalamus word gemoduleer deur die retikulêre netwerk (FR), hipokampus, septum en *corpus striatum* as gevolg van wedersydse verbindings veral tussen die hipotalamus, hipokampus, septum en tegmentum[8].

Die noradrenergiese bane in die brein begin hoofsaaklik in die breinstam en loop dan na die hipotalamus, skors, hipokampus en serebellum via die MFB en septum[4]. Die dopamien bane begin hoofsaaklik in die *substantia nigra* in die omgewing van die basale kerne en loop dan deur die laterale hipotalamus na die basalekerne[4]. Sommige dopamien bane het egter hulle ontstaan in die hipotalamus en loop vervolgens tot in die eminentia medianus waar hulle eindig[4 12]. Dit is onlangs ook gevind dat die hoogste konsentrasie noradrenalien in die hipotalamus en *eminentia medianus* voorkom, terwyl die hoogste konsentrasie dopamien ook in die *eminentia medianus* voorkom[4]. Vir volledigheidshalwe word die biosintese van noradrenalien en dopamien in figuur 3 weergegee.

Verder ontvang die hipotalamus ook noradrinergiese vesels vanaf die pons en medulla oblongata[12] en serotoninergiese vesels vanaf die pre-optiese area. Laasgenoemde bane loop deur die MFB na die hipotalamus[12 37 48 52]. Dan is daar ook nog die baie belangrike simpatiese vesels beskrywe wat vanaf die boonste servikale ganglion na die hipotalamus loop[27].

Soos later vemeld sal word, is die pineale klier baie gevoelig vir ligprikkels. In die laer vorme is die pineale klier self in staat om die ligprikkels waar te neem[18] maar in die soogdier is dit nie meer die geval nie[52]. Ten spyte hiervan is die klier steeds 'n belangrike skakel in die waarnemingsketting van ligprikkels. Daar is egter geen bewyse in die literatuur of 'n beskrywing van 'n direkte baan tussen die retina en hipotalamus[33 52] of tussen retina en pineale klier nie[52]. Daar is egter goeie bewyse dat ligprikkels die pineale klier langs die volgende weg in soogdiere bereik, nl. retina, inferior bykomstige optiese baan, MFB, rostrale tegmentum, breinstam, intermediolaterale kern in die boonste torakale rugmurg, boonste servikale ganglion, postsinaptiese noradrenergiese (simpatiese) vesels en pineale klier[3 4 18 21 28 37 48 50]. Die inferior bykomstige optiese baan skei van die primêre of visuele optiese baan net na die optiese oorkruising en loop dan deur die M.F.B.[37 52].

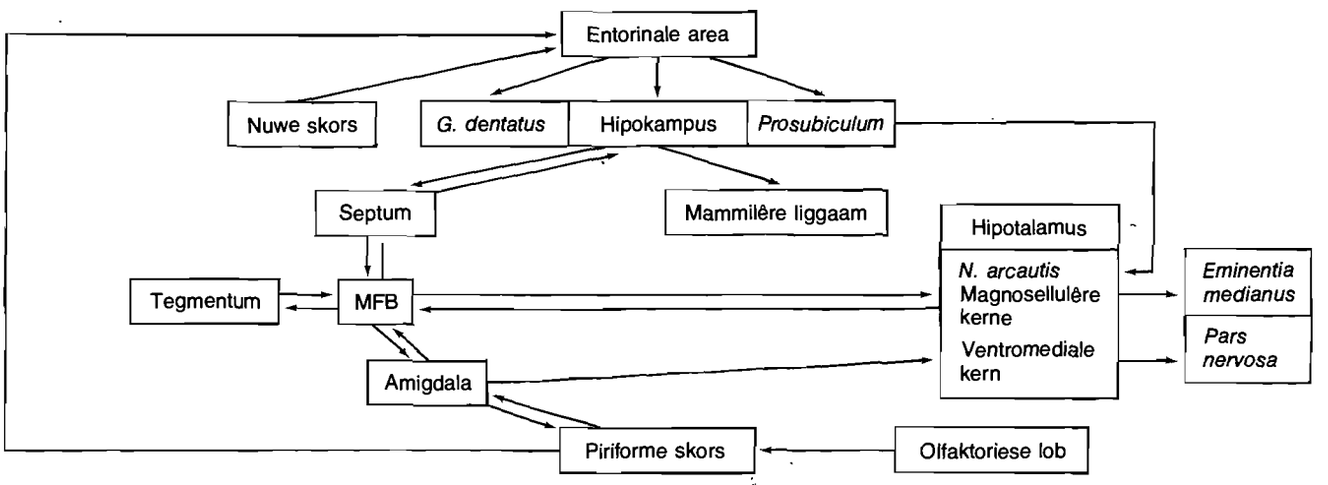
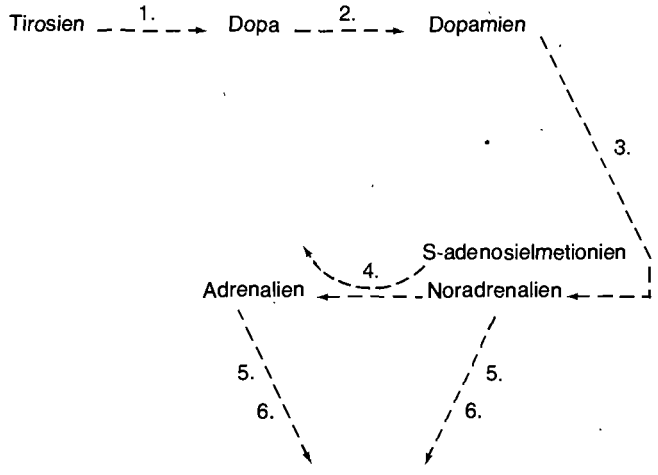


Fig. 2. Hipotalamiese verbindings volgens Raisman[33].



1. Tirosienhidroksilase
2. Aromatiese aminosuurdekarboksilase
3. Dopamienhidroksilase
4. Fenieletanolamien-N-metieltransferase (PNMT)
5. Katesjol-O-metieltransferase (COMT)
6. Monoamienoksidase (MAO)

Fig. 3. Biosintese van dopamien, noradrenalien en adrenalien en die metabolisme van adrenalien en noradrenalien[4].

Alternatiewelik mag die invloed van lig natuurlik ook via die neokorteks, entorinale skors, hipokampus, septum en M.F.B. na die hipotalamus herlei word[33]. Die inferior bykomstige optiese baan kan egter ook ligprikkels direk na die laterale hipotalamus voer via die M.F.B.[52].

In die geval van voëls speel die oë nie die enigste rol in die oordraging van ligprikkels na die pineale klier nie. Dit wil voorkom asof 'n ander orgaan hierby betrokke moet wees[18 37] en dele wat wel genoem word, is die Harderse klier rondom die oog en die direkte invloed van lig wat deur die skedel straal[3].

Die oordraging van simpatiese senuweeprikkels in die pineale klier geskied deur die afskeiding van serotonien en noradrenalien[51] wat dan as chemiese geleiers funksioneer. Dit is veral noradrenalien wat as die belangrikste chemises geleier beskou word[4 52].

Vir volledigheidshalwe word hier ook 'n kort uiteensetting gegee van die tipe innervering wat bekend is om spesifieke hormone vanuit die hipotalamus vry te laat. So is dit bekend dat noradrenalien (d.w.s. noradrinergiese bane) verantwoordelik is vir die vrylating van LH - VH[5 12 35] en ook 'n rol speel by die vrylating van FSH - VH, TSH - VH en GH - VH. Laasgenoemde twee vrylatingshormone is ook onder dopamien beheer[12 35].

Die afskeiding van prolaktien word gestimuleer deur serotonien en geïnhibeer deur dopamien[35]. Dopamien kan egter ook 'n rol speel in LH -VH afskeiding[5 25] en serotonien mag ook 'n rol speel in die afskeiding van FSH, LH en prolaktien[22]. Cholinergiese vesels is oor die algemeen stimulerend vir ACTH - VH afskeiding[35] en inhiberend vir FSH - VH en LH - VH afskeiding[6]. Die beheer oor vrylatingshormone word egter deur 'n groot getal faktore uitgeoefen waarvan olfaksie, die limbiese stelsel, serotoninergiese en noradrinergiese bane die belangrikste dele uitmaak[52].

PINEALE EVOLUSIE

Die pineale klier van visse en amfibiese diere is 'n sensoriese orgaan wat in staat is om ligprikkels vanself waar te neem[18], met ander woorde hierdie klier funksioneer as 'n fotoreseptiewe orgaan in hierdie spesies. Hierbenewens kan hierdie ligprikkel dan ook omgesit word in 'n senuwee impuls[52] en sekretoriese reaksie[18]. In die reptiele en voëlsoorte is die fotoreseptiewe funksie baie minder opmerklik en is die pineale kliere van hierdie spesies meer sekretories van aard[18]. In beide hierdie spesies kom daar egter uitsonderings op die reël voor waarop daar nie nou in besonderhede hoef ingegaan te word nie.

Die pineale klier van soogdiere het in die proses van ontwikkeling egter sodanig verander dat dit nie meer betrokke is by die proses van fotoresepsie nie[52] maar die klier is steeds liggevoelig[18 21]. Die fotoreseptiewe apparaat van die vis en amfibiese pineale klier word in die soogdier klier verteenwoordig deur die pinealosiet wat nou geheel en al 'n sekretoriese funksie het[18 31]. In hierdie proses van evolusie word die sekretoriese funksie dus algaande sterker en kom daar ook in 'n toenemende mate 'n beter outonomiese en wel 'n noradrinergiese simpatiese innervering van die pineale klier voor[18]. Die pineale klier van die soogdier kan dus beskou word as 'n fotosensitiewe neuro-endokriene orgaan[18].

Die sekretoriese eienskap van die pineale klier sal in 'n latere afdeling meer breedvoerig bespreek word. Die geïnteresseerde leser word na hoofstukke deur Collin[7], Kappers[18] en Oksche[31] verwys vir 'n meer breedvoerigebespreking oor pineale evolusie.

PINEALE BIOCHEMIE

Die belangrikste biochemise kenmerk van die pineale klier is die feit dat die ensiem hidroksieindool-O-metieltransferase (HIOMT) wat verantwoordelik is vir die sintese van melatonien, uitsluitlik hier gevind word[48 49 52 53]. Gevolglik kan melatonien slegs in die pineale klier gevorm word en wel onder simpatiese beheer[28]. Onlangse bevindings dui egter daarop dat die ensiem ook in die retina en in die Harderse klier rondom die oog van rotte voorkom[36].

Hierbenewens kom daar ook verskeie ander biochemiese stowwe in die pineale klier voor, nl. noradrenalien, serotonien, 5-hidroksieindoolasynsuur (5 HIAA), 5-metoksieindoolasynsuur (5 MIAA), 5-hidroksietriptofol, 5-metoksietriptofol en 5-hidroksietriptofaan. Verskeie ensieme word ook hier aangetref benewens die redes gemeld, nl. triptofaanhidroksilase, aromatiese aminosuurdekarboksilase, N-asetieltransferase, monoamienoksidase en sikliese adenielmonofosfaat (sAMP)[3 4 28 36 44 48 52 53].

Die biosintetiese en metaboliese pad van melatonien word in fig. 4 weergegee. Figure 5 en 6 dien ter illustrasie van die spesifieke sellulêre struktuur wat betrokke is in die biosintese van melatonien in die pineale klier. Die spesifieke rol van noradrenalien in die biosintese van melatonien word in fig. 7 weergegee.

Dit is verder belangrik om op sekere ritmes te let wat daagliks in die pineale klier voorkom. Halberg[14] beskryf twee belangrike ritmes soos volg:

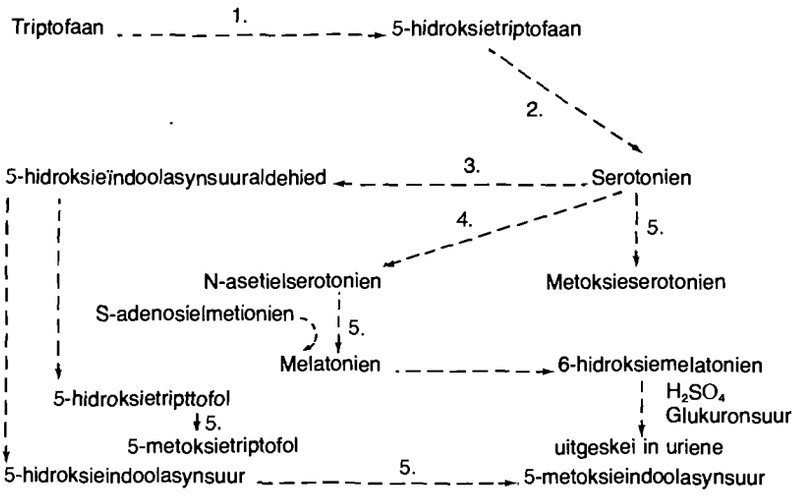
(a) 'n Etmaalritme (circadian) wat 'n fisiologiese periode is van 24 uur maar soms ook periodes wat egter konstante hoeveelhede korter of langer as 24 uur is.
(b) 'n Daaglikse ritme (diurnal) wat dui op 'n ritme binne die daglig periode.

Daaglikse ritmes word na alle waarskynlikheid beïnv-

loed deur adrenokortikale, ovariese steroïed en hipofiseale hormone terwyl cholinergiese en serotoninergiese bane 'n belangrike skakel vorm in hierdie netwerk. Hierbenewens speel die hipotalamus en limbiese stelsel asook die lig- donker siklus 'n baie belangrike rol[34].

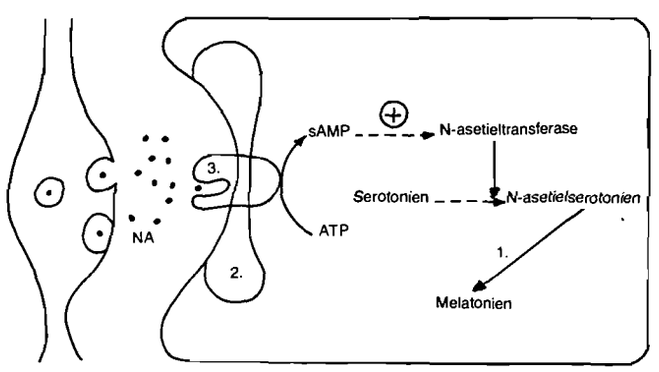
Die volgende biologiese ritmes is reeds in die pineale klier aangedui met vermelding van die biochemiese stof daarby betrokke en stimulus daarvoor verantwoordelik:

(a) 'n Daaglikse ritme vir noradrenalien met hoë vlakke tydens die ure van donkerte[3 4 36 52].
(b) 'n Etmaalritme vir serotonien met hoë vlakke gedurende die dagligure[3 4 36]. Hierdie ritme is direk verbind met die simpatiese stelsel[4] maar kan nogtans in stand gehou word in blinde en/of geblindoekte diere[52].
(c) 'n Etmaalritme vir die ensiem N-asetieltransferase met piek vlakke tydens die ure van donkerte, d.w.s.180° uit fase met die ritme van serotonien[3 4 36].



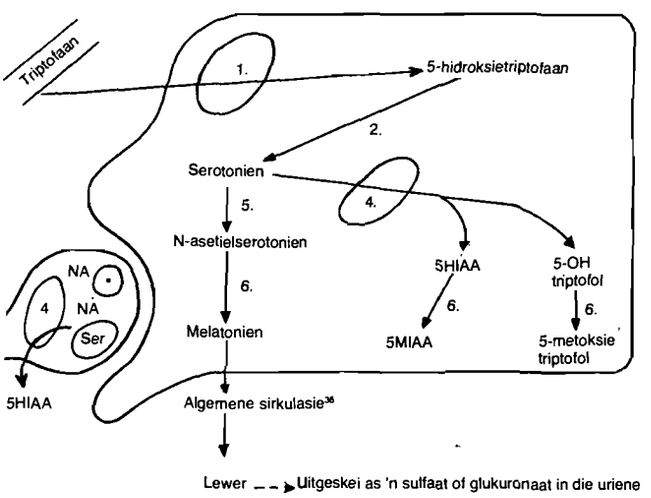
1. Triptofaanhidroksilase
2. Aromatiese aminosuurdekarboksilase
3. Monoamienoksidase (MAO)
4. N-asetieltransferase
5. Hidroksieïndool-O-metieltransferase (HIOMT)

Fig. 4. Die biosintese en metabolisme van melatonien soos beskryf deur verskeie outeurs[3 4 28 32 36 43 48 52 53]



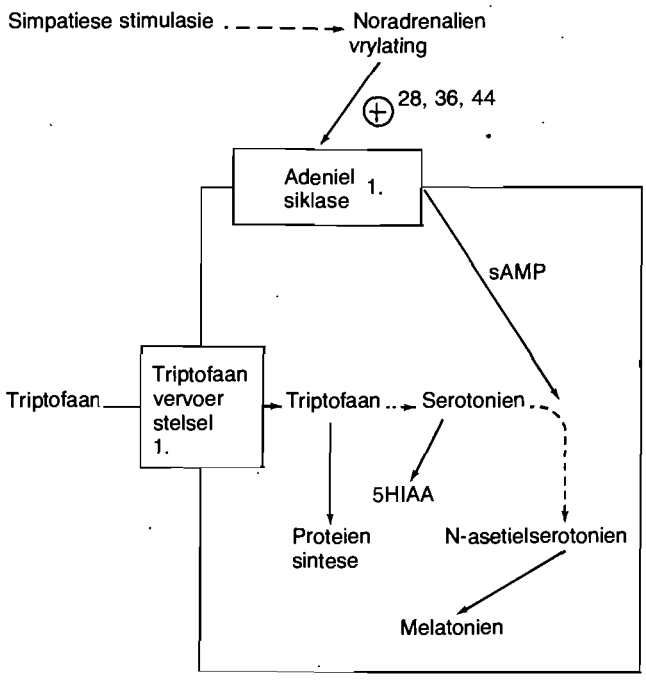
1. HIOMT
2. Adeniel siklase
3. β reseptor
4. NA = Noradrenalien

Fig. 5. Die biosintese van melatonien: die rol van sAMP[4].



1. Mitochondrion met triptofaanhidroksilase
2. Aromatiese aminosuurdekarboksilase
4. Mitochondrion met monoamienoksidase
5. N-asetieltransferase
6. HIOMT

Fig. 6. Biosintese en metabolisme van melatonien: sellulêre organisasie[53].



1. β reseptor[19 44].

Fig. 7. Die rol van noradrenalien in die biosintese van melatonien volgens Shein[44].

(d) 'n Etmaalritme vir N-asetielserotonien met 'n piek tydens die ure van donkerte[4].
(e) 'n Daaglikse ritme vir melatonien[4 28 36] met piek vlakke tydens die ure van donkerte.
(f) 'n Etmaalritme vir die ensiem HIOMT met hoogste vlakke tydens die ure van donkerte in 'n 12 uur donker/ 12 uur lig etmaal[36 52].

Hierdie ritmes word veroorsaak deur 'n stimulasie van die suprachiasmatiese kern (d.w.s die simpatiese stelsel) deur die toetrede van donkerte en die gevolglike styging van noradrenalien vlakke in hierdie kern[52]. Die noradrenalien aktiveer dan die β-reseptore en die adeniel siklase stelsel met gevolglike produksie van sAMP. Laasgenoemde stimuleer N-asetieltransferase wat serotonien omsit in N-asetielserotonien[3 4 28 44]. Indien 'n ligprikkel in die nag toegedien word, verlaag dit die noradrenalien vlakke met 'n gevolglike daling in die N-asetieltransferase aktiwiteit omdat die β-reseptore dan meer onbeset is[4]. Die noradrenalien wat tydens donkerte afgeskei word, stimuleer ook die ensiem HIOMT wat verdere funksies vervul in die instandhouding van die biologiese ritmes[28].

Die biochemiese gevolge in die pineale klier nadat impulse van donkerte ontvang en verwerk is, sluit die volgende in, nl.
(a) noradrenalien afskeiding[3 4 36 52]
(b) verhoogde triptofaanhidroksilase aktiwiteit[52]
(c) verhoogde N-asetieltransferase aktiwiteit[3 4 36]
(d) N-asetielserotonien afskeiding[4]
(e) verhoogde HIOMT aktiwiteit[28 36 52 53]
(f) Melatonien afskeiding[4 28 36 52]
(g) verlaagde serotonien aktiwiteit[3 4 28 36 52]

Ligprikkels het oor die algemeen die teenoorgestelde biochemiese gevolge in die pineale klier nl.
(a) verlaagde noradrenalienaktiwiteit[4 28 48]
(b) verlaagde N-asetieltransferase aktiwiteit[4 51]
(c) verlaagde N-asetielserotonien aktiwiteit[4]
(d) verlaagde HIOMT aktiwiteit[3 10 18 28 48 51-53]
(e) Melatonien inhibisie[10 28 52]
(f) verhoogde serotonien aktiwiteit[3 4 28 36 52]

Benewens die inloed van lig en/of donkerte op die biochemiese aktiwiteit van die pineale klier is daar ook bewyse dat sekere ander faktore 'n rol hierin kan speel. So is daar bv. gevind dat lokomotoriese aktiwiteit 'n definitiewe rol speel en 'n stimulerende invloed uitoefen op melatonien afskeiding. Hierdie stimulasie veroorsaak ook 'n ritmiese afskeiding van melatonien weens die etmaalritme in lokomotoriese aktiwiteit[36]. In toestande van langdurige beligting vind die teenoorgestelde egter plaas met 'n verhoging in N-asetieltransferase en melatonien slegs na immobilisasie[24].

Dit is ook gevind dat kopulasie (en dus indirek lokomotoriese aktiwiteit) 'n rol speel in die beheer van HIOMT-peile[46] en dat spanning (stress) 'n styging teweegbring in die peile van N-asetieltransferase[28]. Hierbenewens is dit ook reeds beskryf dat stress[18], temperatuur[27 37], gehoor[37] en voeding[37 38] 'n rol kan speel in die beheer van pineale metaboliese aktiwiteit. Neurale verbindings tussen die olfaktoriese strukture en die epitalamo – epifiseale stelsel is ook reeds beskryf[37] en mag dus ook betrokke wees by die beheer van pineale metaboliese aktiwiteit.

Sommige van hierdie faktore word in figuur 8 uitgebeeld. Die geïnteresseerde leser word verder erwys na verskeie publikasies waarin die aspek van biologiese ritmes meer breedvoerig bespreek word[5 16 34 36 41] asook na 'n oorsigsartikel oor die effek van lig op die pineale klier en geslagsklierfunksie[51].

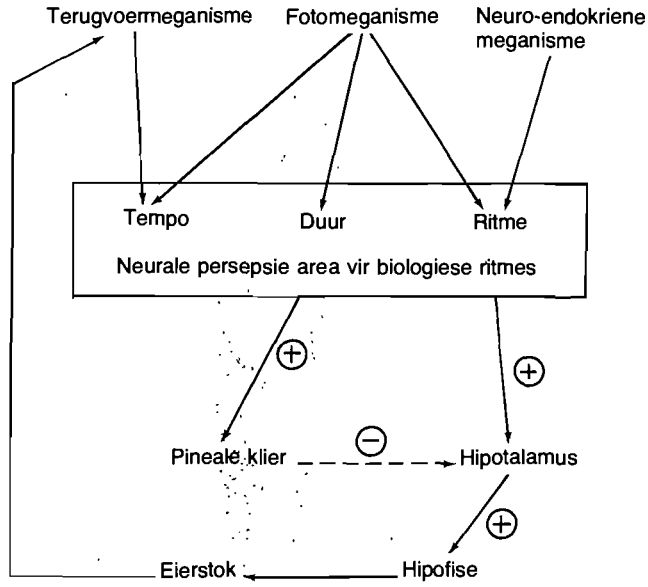


Fig. 8. Beheer van pineale metaboliese aktiwiteit volgens Yochim & Wallen[54].

PINEALE FUNKSIES

Die presiese funksie van die pineale klier is vandag nog steeds nie bekend nie. By die laer vorme en by verskeie laboratoriumdiere bestaan daar wel bepaalde idees in hierdie verband, maar by die soogdier en spesifiek by ons plaasdiere is daar weinig bekend oor die spesifieke rol wat die pineale klier speel in die homeostase van so 'n dier. Die funksies van spesifieke stowwe wat in die

pineale klier voorkom, sal in die volgende hoofstuk behandel word. Die juiste meganisme van aksie van die pineale sekresies is ook nog grootliks onbekend maar een moontlikheid wat genoem word is die aktivering van adrinergiese bane en 'n gevolglike inhibisie van die hipofiseale – adrenale as[30].

Oor die algemeen is dit bekend dat die pineale klier 'n invloed uitoefen op die geslagskliere, tiroïed, hipofise, gladdespier, slaappatroon en serotonien inhoud van die brein[52]. Dit oefen 'n anti-gonadotrofiese effek[24 28 29 37] uit op SSS – vlak[37] en beïnvloed verder ook geslagtelike rypwording[11]. Daar is ook enkele bewyse van adrenaal onderdrukking as gevolg van pineale aktiwiteit[37].

Daar is ook verskeie outeurs wat die hipotese stel dat die pineale klier 'n biologiese ritme bepaal vir ander organe[10 52 53] en dat die suprachiasmatiese kern in hierdie verband 'n belangrike rol speel[4]. Die pineale klier word verder ook beskou as 'n neuro-endokriene geleier wat senuwee impulse (NA), omsit in endokriene stowwe, nl. melatonien en ander metoksie-indool stowwe soos 5-metoksietriptofol en 5-metoksieindool asynsuur[48]. Verder is die funksie van die pineaal ook al beskryf as synde betrokke te wees by seisoenale voortplanting waar dagliglengte 'n spesifieke rol speel[29 36 52].

Die antigonadotrofiese effek van die pineale klier word grootliks teweeggebring deur omgewingsbeligting[4 10 21 37 52]. So is dit bekend dat konstante beligting in rotte aanleiding gee tot aanhoudende estrus[28 37] met lae pineale vlakke van HIOMT[18] en dat langdurige donkerte aanleiding gee tot negatiewe effekte op die voortplantingsfunksies van sodanige rotte. In primate is die toestand egter omgekeerd aangesien aanhoudende beligting lei tot hoë HIOMT-peile in ape[18]. In die geval van hamsters is gevind dat aanhoudende beligting nie aanhoudende estrus tot gevolg het nie[42] maar dat addisionele beligting in die lente tot die voorkoms van estrus verhaas[17].

Definitiewe antigoadotrofiese effekte is ook reeds in die pineale klier van beeste beskryf. In een geval word melding gemaak van ovariese atrofie in rotte na toediening van bees pineale ekstrakte[28]. In 'n weefselkultuur van hipofise en hipotalamus het Hayes, Knight & Warton[15] gevind dat byvoeging van bees pineale weefsel lei tot 'n onderdrukking van LH vorming. In 'n poging om 'n vroeëre bevinding van groot hoeveelhede Gn-VH in die pineale klier van beeste te bewys, kon Gradwell, Millar & Symington[13] nie daarin slaag nie en het hulle slegs 'n klein fraksie LH - VH in die pineale klier kon bind van dié wat normaalweg in die hipotalamus aangetref word.

Een verdere aspek van pineale funksie wat nog toegelig moet word, is die gevolge van pinealektomie en dit sluit o.a. die volgende in:

(a) Stimulasie van FSH en LH[10 37]. Een groep werkers gaan selfs sovêr as om pinalektomie 'n LH-mobiliserende faktor te noem[26]
(b) Verhoging van tiroïed gewig[37 52]
(c) Eierstok hipertrofie[10]
(d) Vaginale keratinisasie in knaagdiere[10]
(e) Testikulêre hipertrofie[10]
(f) Vergroting van die prostaat en seminale sakkies[10]
(g) Verlaging van hipotalamiese serotonien peile[29] (Serotonien anti-gonadotrofies[29] en veral anti-FSH[10 11 37])
(h) Voorkoming van geslagsklier atrofie in blinde hamsters[42]
(i) Voorkoming van vroeë estrus in hamsters na addisionele beligting in die lente[17].

'n Laaste aspek van pineale fisiologie wat nog gemeld moet word, is die effek van pineale gewasse. Dit is bekend dat daar twee tipes pineale gewasse voorkom, en dat hulle verskillende simptome tot gevolg het. Parenkiem gewasse vermeerder gewoonlik die HIOMT-inhoud van die klier en lei tot vertraagde puberteit terwyl gewasse van die stroma of terratomas aanleiding gee tot vroeë puberteit[23].

Die geïnteresseerde leser word verder na verskeie publikasies verwys vir breedvoerige oorsigsbesprekings van pineale biochemie en fisiologie[1 28 32 37 44 47 52 53].

PINEALE ENDOKRINOLOGIE

Alhoewel verskeie pineale funksies reeds in die vorige hoofstuk genoem is, is dit tog nodig om in meer detail op sommige van die funksies te let veral in die gevalle waar spesifieke funksies aan spesifieke pineale stowwe toegeskryf word. Verskeie sodanige stowwe is al deur verskeie outerus beskryf as synde die aktiewe sekretoriese produk van die pinealosiet met hier en daar sprake van 'n pineale hormoon of hormone. Die stowwe wat reeds as sodanig beskryf is, is onder andere:

(a) argenien vasotosien[28 32 36 39]
(b) melatonien[2 4 9–11 17 28–30 36 37 39 40 48 51 52]
(c) Ongeïdentifiseerde polipeptied faktore[32 36 37 39 40]
(d) 5-hidroksietriptofol[9–11 30 37 39]
(e) 5-metoksietriptofol[10 11 28 30 36 37 39]

Die verskillende funksies wat reeds vir hierdie stowwe beskryf is, is omvangryk en sal hieronder weergegee word onder afsonderlike hoofde.

Argenien Vasotosien

(a) Negatiewe effekte op die eierstokke, testes by bykomstige geslagsorgane in rotte[28].
(b) Negatiewe effekte op voortplanting veral in knaagdiere[39].

Melatonien

Hierdie chemiese stof is volgens verwysing vir die eerste keer in 1958 deur Lerner en medewerkers beskryf[28 37]. Biochemies gesproke is dit een van die metoksie-indool stowwe en wel N-asetiel-5-metoksietriptamien[37] wat uitsluitlik in die pineale klier gesintetiseer word. (sien die hoofstuk oor pineale biochemie). Nadat dit gevorm is, word dit of in die serebrospinale vloeistof afgeskei[21 52] of in die bloedstroom opgeneem[18 35]. Die halfleeftyd van melatonien in die sirkulasie is baie kort[52] en duur slegs sowat 25 minute[21]. Melatonien word geredelik vanuit die bloedstroom deur die brein opgeneem[48] en dit konsentreer in die hipotalamus[2 48] en middelbrein[2 37 48]. Sover bekend is daar geen bewyse vir pineale metabolisme van melatonien nie[52].

Die biologiese effek van melatonien in die liggaam behels 'n wyd uiteenlopende reeks reaksies en sluit die volgende belangrikste reaksies in, nl.

(a) Onderdrukking van voortplantingsfunksies in meeste spesies met die uitsondering van hamsters[37 39 53].
(b) Onderdrukking van die geslagkliere, dus 'n antigonadotrofiese effek[36 48 52].
(c) Onderdrukking van endokriene kliere[48 53]

(d) Onderdrukking van die tiroïed [28 37 48] met gevolglike kropgeswelverwekkende eienskappe[37].
(e) Onderdrukking van hipofiseale funksie[28 48 52]
(f) Vertraging van puberteit[11 37]
(g) Vermindering van eierstok gewigte[4 28 37], testikulêre gewig[4 28] en geslagsklier gewigte[28 37].
(h) Onderdrukking van gladdespier sametrekking[28 52 53]
(i) Inhibisie van ovulasie[11 28]. Hierdie effek kan wees as gevolg vn 'n onderdrukking van LH of as gevolg van 'n intrinsieke sedatiewe effek van melatonien[11]. (sien ook die volgende twee punte)
(j) Induksie van slaperigheid[10 28 48 53]
(k) Onderdrukking van LH afskeiding[4 11 28 37 51]. Hierbenewens is daar verskeie verwysings na die onderdrukkende effek van melatonien op LH afskeiding na implantasie in die *eminentia medianus*[4 9-11 37 48] middelbrein[2 9 11 52] en na intraventrikulêre toediening[10 11].
(l) Onderdrukking van plasma kortikosteroon[30].
(m) Onderdrukking van testosteroon in die testikulêre veneuse plasma[28].
(n) Onderdrukking van GH afskeiding[28].
(o) Onderdrukking van Gn-VH[28].
(p) Verhoging van serotonien in die middelbrein[2 37 48 52].
(q) Oor die algemeen blyk dit dat melatonien geen effek het op FSH-peile nie[10 37]
(r) Verhoging van brein GABA[2] (kan funksioneer as chemiese oordraer van senuwee-impulse)
(s) Verhoging van brein piridoksal kinase wat 'n belangrike rol speel in PO_4-oordraging in die sintese van dopamien (DA), serotonien, GABA en aromatiese aminosuurdekarboksilase[2].
(t) Onderdrukking van estrus in rotte wat in konstante lig aangehou word[4].
(u) In teenstelling met die onderdrukkende funksies soos hierbo genoem, is daar ook enkele bewyse van 'n stimulerende effek van melatonien op voortplantingsfunksies in die algemeen[39 40]. Hierdie teenstrydigheid word egter gedeeltelik verklaar deur die feit dat die lig/donker siklus 'n groot rol speel by die bepaling van in inhiberende of stimulerende effek van melatonien[39].

Polipeptied faktore

(a) Hierdie faktore het blykbaar 'n antigonadotrofiese effek[23 26] met 'n onderdrukkende invloed op voortplanting[40].
(b) Indien hulle wel antigonadotrofies is, dan speel die indool amiene moontlik 'n rol in hulle sintese en/of vrylating[39].

5-Hidroksie-triptofol

(a) Speel 'n rol in die beheer van LH afskeiding en oefen 'n negatiewe effek uit op LH vlakke[37].
(b) Implantasie in die *eminentia medianus* lei tot verlaagde LH vlakke[9-11]. Dieselfde reseptore as in die geval van melatonien is hierby betrokke[37].
(c) Antigonadotrofiese effekte[39].
(d) Verlaging van plasma kortikosteroon[30].

5-Metoksie-triptofol

(a) Onderdrukking van geslagskier aktiwiteit[36].
(b) Vertraging van puberteit[11].
(c) Verlaging van eierstokgewigte[28].
(d) Verlaging van FSH na implantasie in die *eminentia medianus*[10 11 27]. (Dit geld ook vir serotonien[10 11 29 37].) Hierdie effek word veroorsaak na binding aan 'n spesifieke reseptor wat met serotonien gedeel word[10 37].
(e) Geen effek op LH[37] alhoewel 'n verlaging van hipofiseale LH al beskryf is[28].
(f) Vermindering van plasma kortikosteroon[30].
(g) Oor die algemeen meer potent as melatonien met dieselfde spektrum van biologiese effekte[39].

Hierbenewens is daar ook definitiewe bewyse van 'n hormonale[4 36] invloed op die biosintese van melatonien en ander pineale produkte. So is dit byvoorbeeld bekend dat estrogene onder eksperimentele toestande 'n inhiberende invloed uitoefen op die pineale klier[37] en veral op die vlakke van HIOMT[28 32 46] en N-asetieltransferase[32]. Gevolglik onderdruk dit ook die biosintese van melatonien[32]. Daar is egter verhoogde proteien sintese in die pineale klier onder estrogeen stimulasie[28] maar nogtans is HIOMT-vlakke laer tydens estrus as tydens met-en di-estrus[32 52 53]. Verder blyk dit egter asof fisiologiese dosisse van estrogeen HIOMT sintese in die pineale klier stimuleer[32] terwyl hoë dosisse die teenoorgestelde effek het[32]. Daar is goeie bewyse vir die teenwoordigheid van *'n* spesifieke hoë affiniteit – lae kapasiteit sitosol reseptor vir estrogeen in die pineale klier[32].

Oor die effek van progesteroon op pineale aktiwiteit is daar baie min bekend en dit wil oor die algemeen voorkom asof spesie verskille 'n baie groot rol speel[32]. Sover bekend is die enigste effek 'n onderdrukking van HIOMT aktiwiteit[32]. Oor die algemeen is 'n hoë dosis testosteroon ook inhiberend ten opsigte van HIOMT[28 32] terwyl 'n fisiologiese dosis stimulerend is[32]. Geen effek van testosteroon kon nog gevind word op die konsentrasie van N-asetiel-transferase nie[32]. Soos in die geval van estrogene bestaan daar 'n spesifieke hoë affiniteit – lae kapasiteit sitosol reseptor vir testosteroon in die pineale klier[32]. Een ander belangrike aspek van androgeen invloed op die pineale klier is die metabolisme van androgene in die pineale klier na estrogene[32].

Daar is verder weinig bekend oor die invloed van die hipofise op pineale aktiwiteit. In hierdie verband moet die indirekte rol van steroïede egter nie verontagsaam word nie[32]. Oor die algemeen word dit egter aanvaar dat simpatiese oordaging 'n belangrike rol speel by die effek van hormone op die pineale klier[28].

GEVOLGTREKKING

Uit die voorafgaande is dit dus heel duidelik dat die pineale klier primêr deur die lig – donker siklus beïnvloed word[4 10 21 37 52] en dat donkerte primêr verantwoordelik is vir die metaboliese aktiwiteit van die pineale klier[3 4 28 36 52 53] en gevolglike sintese van melatonien[4 28 36 52] terwyl lig oor die algemeen 'n onderdrukkende effek het op pineale aktiwiteit[3 4 10 18 28 48 51 52]. Dit is ook duidelik dat die pineale stowwe oor die algemeen 'n primêre negatiewe invloed uitoefen op verskeie aspekte van die voortplantingssiklus van die vroulike dier[2 4 9 11 24 28 29 36 37 40 48 51-53].

Aangesien meeste eksperimentele werk in hierdie verband op laboratoriumdiere soos muise en rotte gedoen word, is dit uiters moeilik om hierdie resultate op die huisdiere van toepassing te maak. Slegs in die pine-

ale klier van die bees is daar tot op datum antigonadotrofiese stowwe beskryf[15 28].

'n Belangrike aspek by die bepaling van pineale funksie, is die bestaan van spesie verskille soos bv. tussen rotte aan die een kant en ape en hamsters aan die ander kant[17 18 28 37 42]. So mag daar ook definitiewe verskille bestaan tussen die verskillende huisdier spesies ten opsigte van hulle pineale reaksies in verskillende lig – donker siklusse.

Dit wil egter wel voorkom asof die pineale klier wel met die voortplantingsfunksies van diere gekoppel is, maar in welke mate en in welke hoedanigheid is op hierdie stadium nog grootliks onbekend. Dit is egter reeds geopper dat die pineale klier wel betrokke mag wees in spesies waar seisoenale teling voorkom en waar dagliglengte 'n spesifieke rol speel[29 36 52].

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ADDENDUM

Sedert die skrywe van hierdie oorsig het verdere inligting onder die skrywer se aandag gekom ten opsigte van die verspreiding van vrylatingshormone en chemiese geleiers in die *eminentia medianus* van die bees[1] en die invloed van omgewingsbeligting op die serum melatonien peile van skape.[2]

Wat die verspreiding van LH - VH betref in die *eminentia medianus* van die bees, is gevind dat LH - VH veral voorkom in die anterior binneste en middel buitenste dele van die *eminentia medianus*. In hierdie gebiede was slegs dopamien en asetielcholien teenwoordig in betekenisvolle hoeveelhded, en die afleiding word dus gemaak dat slegs hierdie twee geleiers betrokke is by LH - VH afskeiding, vanuit die bees *eminentia medianus*[1].

In 'n ander publikasie word bewyse gelewer van 'n etmaalritme vir melatonien in die serum van skape tydens 'n 12 uur lig/12 uur donker etmaal met lae vlakke tydens die lig periode en 3x hoër vlakke tydens die donker periode. In aanhoudende beligtingstoestande is die ritme opgehef met lae melatonien vlakke ongeveer soos in die lig periode van die 12 uur lig/12 uur donker etmaal[2].

Tydens aanhoudende donker toestande het die ritme voorgeduur met baie hoë pieke tydens die ure wat onder normale toestande ure van donkerte sou wees. Ligprikkels tydens hierdie tye het 'n opmerklike en vinnige daling in melatonien vlakke tot gevolg gehad wat toegeskryf kan word aan die kort ½-leeftyd van melatonien in die algemene sirkulasie[2].

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BOOK REVIEW — BOEKRESENSIE

THE VETERINARY ANNUAL – 18TH ISSUE

C.S.G. GRUNSELL AND F.W.G. HILL, Editors
Scientechnica, Bristol 1978 pp xviii + 309, Figs 54, Tabs 24, Publ. Price £11.50.

The latest Annual maintains the high standards we have come to expect from this publication. A new feature, in addition to the usual division of material on a species basis, is a section of Special Articles. These are four in number (50 pp) on virus teratogens, recent advances in animal husbandry, reproduction and infertility and plasma lipoproteins in health and disease.

Large animal topics featuring cattle, sheep, pigs and horses occupy 122 pages and include papers on bovine leptospirosis, antibody in relation to calf pneumonia, Erysipelothrix polyarthritis in lambs, current sheep disease, ectoparasite control developments in sheep, pig preventure medicine, streptococcal infection in young pigs, changing attitudes to foaling, management of sweet itch (culicoides hypersensitivity) in horses, and the biochemical and clinical aspects of exhaustion in the horse.

Small animal topics (117 pp) are particularly practioner-orientated and include racing and muscle injuries in the Greyhound, a particularly good paper on canine pancreatic disease, ulcerative colitis of the Boxer, canine anal sac disease, the epidemiology of hookworm infection (sic), differential diagnosis of canine nasal discharge, feline dermatoses, cat leprosy (a non-tuberculous skin granuloma), progestogens in cats and dogs and the present state of small animal nutrition. The 37 authors who have contributed to the Annual are to be congratulated on the high quality and usefulness of their joint effort.

R.K.L.

DO COLOURS AFFECT 'NORMAL' BEHAVIOUR OF LABORATORY AND FARM ANIMALS? INSTANTANEOUS CHANGE OF BEHAVIOUR BY PRESENTATION OF RED IN THE PEACH-F

H. D. MEBES

ABSTRACT: Mebes H.D. **Do colours affect 'normal' behaviour of laboratory and farm animals? Instantaneous change of behaviour by presentation of red in the Peach-Faced Lovebird** *Agapornis roseicollis* **(PSITTAFORMES).** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 97 (En) Dept. of Biology, University of Kaiserslautern, P.O. Box 5049, D-675 Kaiserslautern, Germany.

In spite of genetic and environmental standardisation in farm and laboratory animal science, a considerable amount of variation in the results of comparable experiments remains. This presumably depends upon the individual variability within a species, and upon differences of various species in their specific demands to the environment in captivity. Due to a discovery in the Southwest-African lovebird species *Agapornis roseicollis,* used as a conventional laboratory animal in bioacoustic research, the phenomenon of instantaneous fear expressed in a number of different displays in connection with the presence or presentation of red objects is described and compared to earlier observations of instantaneous phobias, aggressions, and preferences towards colours, in other bird species.

Consequently it is suggested that investigation of the chromatophobe and chromatophile behaviour of typical laboratory and farm animals be undertaken to ensure the adequate design of lodging and experimental environments.

INTRODUCTION

There are two main tasks in laboratory animal science. The first is to reduce the variation of biological data in animals for experiments, and the second is the standardisation of environmental conditions[4 16 19]. In spite of extreme genetic and environmental standardisation, a relatively large number of variations and of errors in experimental accomplishment remains. This presumably depends upon the individual variation within a species, and upon the differences of various species in their specific demands to the environment in captivity. Therefore, in order to clarify errors, to reduce variations to a minimum, and to maintain the animal's welfare[17], the behavioural preferences and also aversions (phobias) of each species, domesticated or not, must be known and taken into account when designing a species-specific artificial environment for animal lodging in laboratories or on farms.

Owing to an accidental discovery in the Southwest-African lovebird species *Agapornis roseicollis,* used as a conventional laboratory animal in bioacoustic research[14], the phenomenon of instantaneous fear upon the presentation of coloured objects is described and compared to earlier observations of instantaneous phobias, aggressions, and preferences towards colours in other bird species.

MATERIAL

The Southwest-African Peach-Faced Lovebird *Agapornis roseicollis* (Vieillot, 1817) is a sexually monomorphic bird. Throughout the year the four plumage colours of both sexes, red, green, blue,and black, remain unchanged. The beak is yellow, except in juveniles, which have a black saddle marking on the upper maxilla. *Agapornis* species now show some signs of domestication[11]. The number of individuals observed ranged from one isolated couple and four juvenile siblings to two more groups of six and thirteen birds.

*Presented at the "1st World Congress on Ethology Applied to Zootechnics" (Symposium on the Behaviour of Poultry), Oct 23–27, in Madrid, Spain. Published by permission of the Organizing Committee.

Observations on *Agapornis roseicollis*

Even minor changes in the environment of the captive birds caused their temporary arousal. Only until certain aversions seemed obvious, i.e., when the birds avoided me entering their cage room with unfamiliar objects, did I pay particular attention to their behaviour towards colours in and outside the aviary. After some accidental and then intended presentations of red objects, several behavioural indications of fear – in decreasing order – were observed:

1. Alarm calls of some individuals and hasty escape of the whole group into the upper corner of the aviary.
2. A series of mobbing calls and mobbing, i.e., stretched body and wing-beating without flying away from the perch.
3. Attention calls with an alert posture typical for this species, i.e., stretched and sleeked body with eyes widely opened.
4. Relatively normal behaviour, but always carefully watching and avoiding the environment of the 'dangerous' object.
5. Silent approach-and-withdrawal tendency over a period ranging from ten to fifteen minutes up to two to four hours, until eventually nibbling at the object.
6. One other form of display when a tamed and rather adapted lovebird is confronted with a red object without being able to flee: all feathers, particularly those around the neck, ear coverts, crown and forehead are ruffled, and the bird – in most cases females only – utters short and very low "kch"-sounds. This display marks the turning point from defensive aggression to offensive aggression.

Two more examples may illustrate fearful behaviour towards red objects:

1. A red cup filled with seeds was not touched by juveniles for almost two days.
2. The individuals of three adult groups gave up collecting nest material for several hours, when a sheet of red cardboard was laid on the floor. Eventually, owing to a descent of reactivity level, adaptation took place and the birds approached the object. Green, blue, white or black sheets caused no visible fear; yellow sheets caused some fear and precaution. Thereafter, curiosity and approach-withdrawal behaviour occurred,

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and finally the animals nibbled at the plates. (Quantified experiments are in progress.)

In another experiment, directly following the one before, the red pieces of cardboard held as a bundle were again treated as being potentially dangerous, and the birds fled into the upper corner of the aviary. Green, blue, white, or black bundles caused no visible fear; yellow bundles caused some fear. Preiss[15] observed phobia towards red for the first time, when one of his female lovebirds saw the red finger nails of a visiting friend. He then presented two other red objects to the birds (females) which led to the same behaviour.

Observations on other bird species

1. Colour aversions combined with fear (Chromatophobia).

Heinroth[5] names three songbirds, the Yellow Wagtail, *Motacilla flava,* the Yellowhammer, *Emberiza citrinella,* and the Meadow Pipit, *Anthus pratensis,* and cites von Lucanus' observation on the sulphur-Crested Cockatoo, *Cacatua galerita,* all of which were instantaneously horrified by the presentation of blue. According to experiments of von Toerne[18], blue and violet painted wheat corns induced complete aversion in the pheasant, the domestic chick, the pigeon, and the partridge. According to observations of a colleague (personal comunication) the colours dark-brown or black (clothing!) induce fear in the Red-Rumped Parrot, *Psephotus haematonotus,* and a female canary refused to enter its nest with brown strings of wool in it. Instead, the bird preferred to collect its own light-blue and yellow threads.

2. Colour aversions combined with aggressive threat.

Aversions against red in connection with the releasing of intensive fighting are well known in the domestic cock, in turkey cocks, and in the male robin, *Erithacus rubecula,* in its own territory.

3. Colour preferences (Chromatophilia)

Colours which may cause some 'comfort-feeling' in an individual bird, can be listed for the following species[3]: the Jay, *Garrulus glandarius,* chooses preferably blue; a female Jackdaw, *Corvus monedula,* and a Carrion Crow, *Corvus corone,* preferred grey and black (innate releasing mechanism very probable); Yellow Wagtails, *Motacilla flava,* prefer yellow, and Meadow Pipits, *Anthus pratensis,* green colours; the Australian Bowerbird, *Ptilonorhynchus violaceus,* selects only blue colours, and so does the male of the Bluethroat, *Luscinia s. svecica.* Finally, several species of humming birds are said to prefer red colours[2].

Colour preferences in farm animals like chicks, ducklings, and quails have been investigated[1 6 7 8 9 10 12 13].

CONCLUSIONS

Although exact quantitative evidence is lacking in most cases of chromatophobia summarised above, the identical observations of instantaneousness in the aversive behaviour should be valued positively insofar as they point to the fundamental danger of carelessly planned colour conditions at least for those animals which are – for testing purposes – temporarily outside their familiar colour environment. It is evident that many of the above-mentioned bird species do not serve as typical laboratory or farm animals. There is, however, no reason to believe that chromatophobe or chromatophile behaviour does not exist among these, including also laboratory or farm mammals. It is conceivable that white or light-coloured environments, e.g., walls or white overalls of the laboratory personnel – chosen perhaps for reasons of anthropomorphic imagination of cleanliness and hygiene – may cause either fear, or aggression, or 'comfort-feeling' in domesticated or non-domesticated species, and thus a deviation of their 'normal' behaviour in captivity. Therefore, colour experiments within the field of animal lodging design could throw more light onto the species-specific capability of short term or long term, adaptation to colours, or lack thereof. Amazingly, where study of this nature would have its greatest import, i.e., in the field of pharmacological research, published findings, to my knowledge, do not exist.

With respect to the Peach-Faced Lovebird, at least, an atypically large number of alarm or warning calls is usually found in bioacoustic experiments when foreign red objects are presented. I must confess good fortune in dealing with a species so demonstrative in reacting to experimental stimuli.

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CONFERENCE ON MUSCULAR DYSTROPHY IN ANIMALS HELD IN NEW YORK

A conference on muscular dystrophy and other inherited diseases of skeletal muscle in animals was held in New York City on January 25–27, 1978. The aim of the conference, sponsored by the New York Academy of Sciences and Muscular Dystrophy Association, was to achieve a better understanding of recent advances made in the general field of muscle diseases in animals and a critical appraisal of the work being done in the animal field as it related to similar diseases in man. Conference participants also sought to identify areas where scientific knowledge is incomplete or sketchy and to evaluate techniques being used to study some aspects of animal diseases. Other subjects discussed included muscular dystrophy in hamsters, mice and birds; inherited diseases of muscle in man and animals, and inherited diseases of the nervous sytem in small laboratory animals.

The proceedings of the conference will be published in the Annals of the New York Academy of Science.

Source: National Society for Medical Research Bulletin, April 1978.

DEATH ASSOCIATED WITH INHALING TOXIC GAS FROM LIQUID MANURE

On December 8, 1977, a 16-year-old farm worker collapsed and died while steam cleaning gutters inside a calf barn in Eau Claire, Wisonsin. The apparent cause of death was inhalation of toxic gas,with hydrogen sulfide (H_2S) the probable agent. The source of the gas was decomposing liquid manure that had been agitating for 30 minutes to an hour in a 100,000-gallon tank beneath the barn. The boy had been working inside the barn approximately 30 feet from the tank for about 10 minutes when he was overcome. While trying to rescue him, 2 other workers experienced syncopal episodes-,but they recovered. No animals died during the incident; however, no calves were in the affected area at the time of the workers' exposure.

The farm worker had been in good health. He had no chronic illness, took no medications, and had no history of drug abuse. Autopsy findings were consistent with inhalation of a toxic gas resulting in emesis and aspiration. H_2S was implicated as the causative agent by air tests done under similar conditions 2 days after the incident. The tests showed that hydrogen sulfide concentration at the site of death after 8 minutes of manure agitation were >60 ppm. (NIOSH recommends a maximum exposure concentration of no more than 10 ppm over a 10-minute period. When concentrations reach >50 ppm, evacuation is recommended.) Other gases, such as nitric oxide, nitrogen dioxide, and sulfur dioxide, which have been associated wih death in silos, were not detected. Carbon monoxide was ruled out at autopsy by blood tests, methane was thought not to be present since 2 open flame heaters were in use, and ammonia was considered unlikely to have been present in high concentrations because its odor and irritant properties act as warning signals.

The number of liquid manure systems continues to increase in the United States as farmers become more concerned with the efficient recycling of energy-rich waste. Numerous deaths in swine and beef and dairy animals have been associated with exposure to these systems. Several farm workers have died after entering recently emptied liquid manure tanks or have drowned after falling into full tanks. This death is among the first to occur from the inhalation of gas outside the storage tank.

Several factors appear to have contributed to hazardous conditions on the day of the young man's death:

(1) the manure tank was full, and the conents had been agitating longer than usual before the pumping began.

(2) The barn was inadequately ventilated that day; only 1 of the 5 fans was in use, and then only intermittently, and a westerly wind blew through the only open door.

(3) The calves' high protein diet made the formation of hydrogen sulfide more likely. A number of toxic gases are released from decomposing manure, but hydrogen sulfide, carbon dioxide, methane, and ammonia are of principal concern. H_2S, the most toxic of these, even at low concentrations (10–50 ppm) causes headache, irritation of the mucous membranes and respiratory tract, nausea, and dizziness. With increasing concentrations (<100 ppm) one's sense of smell decreases, and at high concentrations (<1,000 ppm) syncope and death following respiratory paralysis may occur with little or no advance warning.

Preventive measures that may be taken to reduce farm workers' risk include improving ventilation and developing contingency plans for evacuating workers and animals from buildings during agitation of manure. Farm workers who must enter a closed space containing a manure tank should wear self-contained air packs and safety harnesses, and reserve workers should be stationed outside.

Source: Center for Disease Control: Death in farm worker associated with toxic gases from liquid manure system, Wisconsin. Morbidity Mortality Weekly Rep 27:47–48, 1978.

EVALUATION OF TECHNIQUES FOR STUDYING THE ARTERIAL SYSTEM OF THE BRAIN OF DOMESTIC RUMINANTS

M.L. GUERRA-PEREIRA and D.J. COETZER

ABSTRACT: Guerra-Pereira M.L.; Coetzer D.J. **Evaluation of techniques for studying the arterial system of the brain of domestic ruminants.** *Journal of the South African Veterinary Association.* (1979) **50** No. 2, 101 (En) Department of Anatomy, Faculty of Veterinary Science, Onderstepoort, 0110, Republic of South Africa.

The techniques used in investigations undertaken to study the arterial blood supply of the brain of domestic ruminants are described and evaluated. These include various coloured intravascular injections, corrosion preparations, injection of contrast media for angiography and clearing of tissues.

INTRODUCTION

During investigations undertaken by the senior author to establish the pattern of the arterial blood supply of the cerebellum of cattle, sheep and goats, various techniques commonly employed in this field of study were assessed. These included intravascular injection masses for dissection purposes, contrast media for angiography, corrosion and clearing methods to demonstrate the macro amd micro-circulation of the brain. The purpose of this paper is to evaluate the different techniques since the experience gained during the course of the study will be of use to others working in the same field.

MATERIALS AND METHODS

Dyes

Various dyes have been recommended for colouring injection masses, such as carmine[2], vermillion (mercuric sulphide[4 21 25], Prussian blue[8 22] and Indian ink[3 5 9 14 15]. Marini-Abreu used Super Tintalac (Robbialac), an inorganic pigment, available in a wide range of colours, in his study of the arterial supply of human cerebellum[15].

Carmine, vermillion, Super Tintalac and Indian ink are chemically stable substances and give an intense colour to the injection masses, even when added in a very small quantity.

Pressure

To obtain well injected specimens care must be taken to apply sufficient pressure to ensure a constant flow and correct penetration of the solution into the finest vascular divisions. It must not be so high, however, as to rupture smaller vessels. Pressure apparatuses of various designs to ensure constant pressure at different levels are described in the literature[5 10 11 12 18 24]. In this study a bottle pressure apparatus connected to a manometer was used. The pressure was around 300 mm of mercury (± 4 000 Pa). Manual injection of the coloured mixture by means of an automatic Roux syringe produced excellent results. Back pressure on the plunger of the syringe was used as an indication of the completed process. Filling of the conjunctival and of the oral mucous membrane vessels was also an excellent indication of a sufficient pressure and correctly filled arterioles.

Injection masses

Gelatine (Fig. 1.) is one of the most satisfactory substances for injection. It is cheap and easy to obtain and

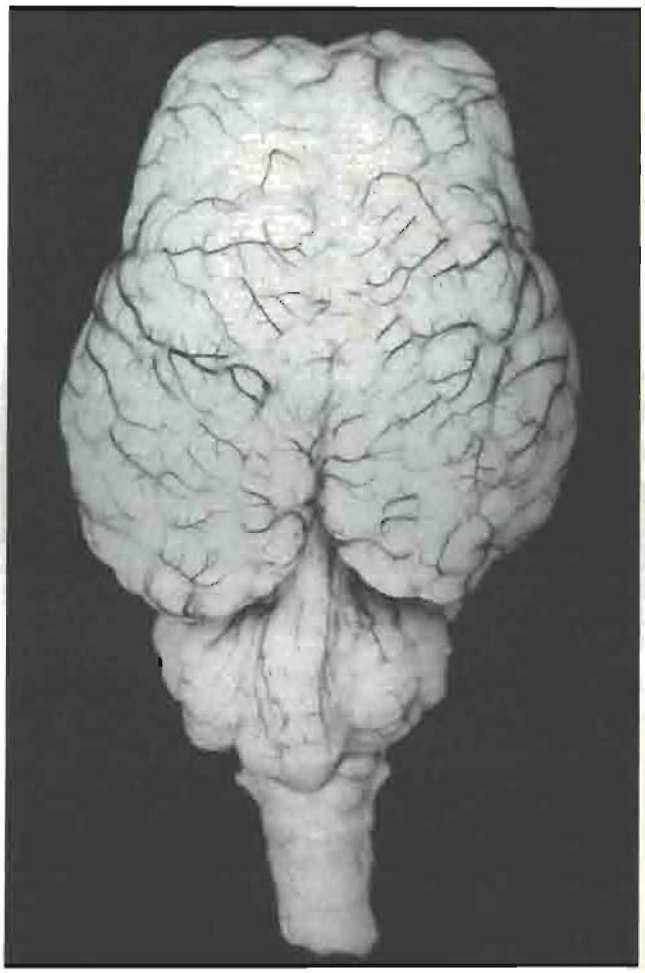

Fig. 1. Brain of the sheep. Dorsal view. Gelatine injection of vessels.

was used extensively in this study. Although various researchers recommend different concentrations[1 2 15 17 25], it was established that a concentration of 5–10 % was the optimum strength for a study of deep vessels and 20–25 % for the superficial vessels. Concentrations exceeding 25 % result in the absorption of too much water after setting with the risk of rupture of the smaller vessels. The injection is made immediately after death and after preliminary flushing of the arterial system with normal saline at a temperature between 40–50 °C. If the specimen to be injected has been allowed to cool down after death its temperature should be raised to body temperature by immersion in warm water before attempting the injection. After the injection the gelatine is allowed to set by placing the specimen (in this case the head of the animal) in a cold

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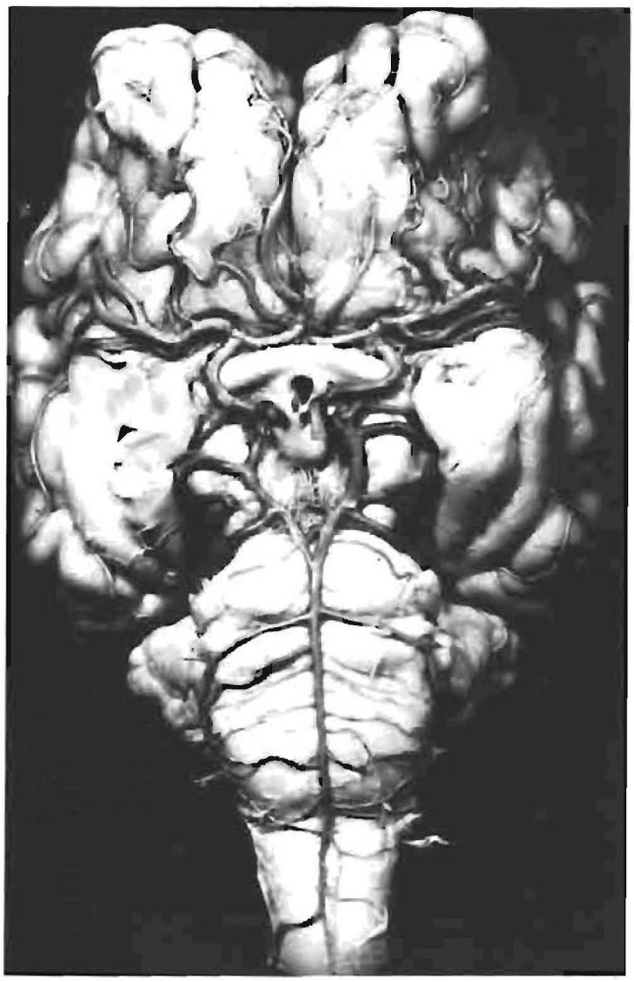

Fig. 2. Brain of the ox. Ventral view. Latex (Revultex) injection of vessels.

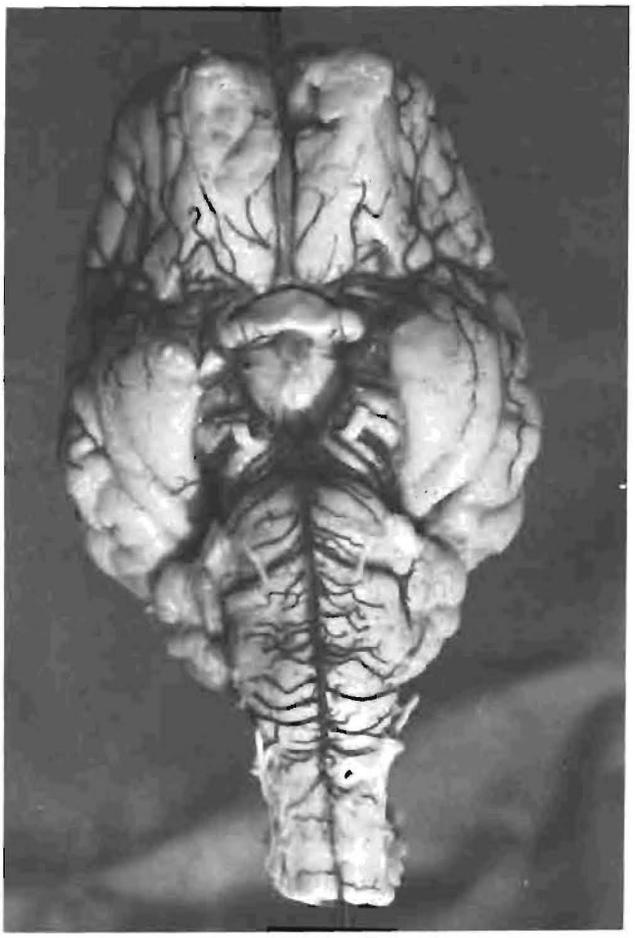

Fig. 3. Brain of the sheep. Ventral view. vinylite injection of vessels.

room for 4–6 h. The brain is then removed and fixed in 10 % of formalin.

Latex* proved to be particularly suitable for arterial injections (Fig. 2.). It can even be injected into preserved specimens and in well blend out subjects it is not essential to flush the arterial system prior to injection. Its toughness and elasticity greatly facilitate dissection. Differently coloured latex suspensions can be obtained by adding dyes (Super Tintalac,** vermillion, ulcanosol red) soluble in alkaline medium. Our best results were obtained by injecting fresh material. Latex can also be used for corrosion preparations.

Agar is not recommended for injection of vessels. It was found to set too quickly, seriously hampering its injection, even in the low concentration of 2 % as recommended by Marques[16]. Vinyl resin (Fig. 3.) produces a rigid cast of the arteries. Vascular beds of excellent quality can be obtained if the vessels are flushed with acetone prior to the injection.

Corosion preparations

The corrosion technique depends on the resistanc. of certain injection masses to macerating fluids such as acetic acid, sulphuric acid, hydrochloric acid, potassium hydroxide, etc. The injection masses found most suitable for corrosion preparations are latex and vinyl resin. It is a valuable addition for studying the pattern of the deeper lying vessels. Vinyl resin (Fig. 4.) has the advantage that the vessel walls are corroded completely leaving a brilliantly coloured cast. It is superior to latex since less shrinkage takes place. After injection the specimen is placed in saline for at least 24 h before maceration. Although concentrated hydrochloric acid has been recommended by various researchers[19] [25] the authors got excellent results by placing the specimens in a 10 % solution of potassium hydroxide.

*"Revultex", Midas Chemicals (Pty) Ltd
**Messrs Robbialac

Roentgenography

Stereo-roentgen-ray examination after injection of radiopaque substances into cerebral arteries and followed by dissection have been found by us to be one of the best methods of studying the gross anatomy of the arteries of the brain. Vinylite acetone solution (6 g of vinylite powder in 60 mℓ of acetone) to which 10 g of red lead powder is added, as recommended by Kaplan[13], produces excellent results (Fig. 5.).

Clearing of tissues

The method of making tissues transparent has been practised for many decades since its introduction by Spalteholz in 1906[23]. It facilitates the study of microcirculation in stereoscopic view over a considerable depth. It is also a cheap and easy technique to perform. In this study the Poulhès modification[20] [21] of the Spalteholz

Fig. 4. Brain of the ox. Ventral view. Vinylite corrosion cast.

technique (Figs. 6, 7.) was applied as follows to sagittal and transverse sections[7] injected with dyed gelatin:

1. The sections were bleached for one week in 3 % hydrogen peroxide. On the first day the liquid was changed at least three times and once on the following days.
2. After bleaching, the sections were washed thoroughly in distilled water for 24 h.
3. The sections were dehydrated in 50, 70, 80, 96 and 100 % ethyl alcohol, allowing them to stay in each for 24 h. With each change the sections were dried between layers of blotting paper.
4. The sections were cleared in pure xylene for 2 or 3 d. During this time, the liquid was changed every 24 h.
5. The clearing was completed in a fluid polyester casting resin*. After 24 h the sections were ready for study and photography. They were handled and photographed while being kept in a petri dish.

Pickworth-Fazio Technique[6]

This technique was also used with commendable results. It is essentially a benzidine and nitroprusside oxi-

*Type GTS, Klaus W. Voss, 2082 Uetersen, Germany.

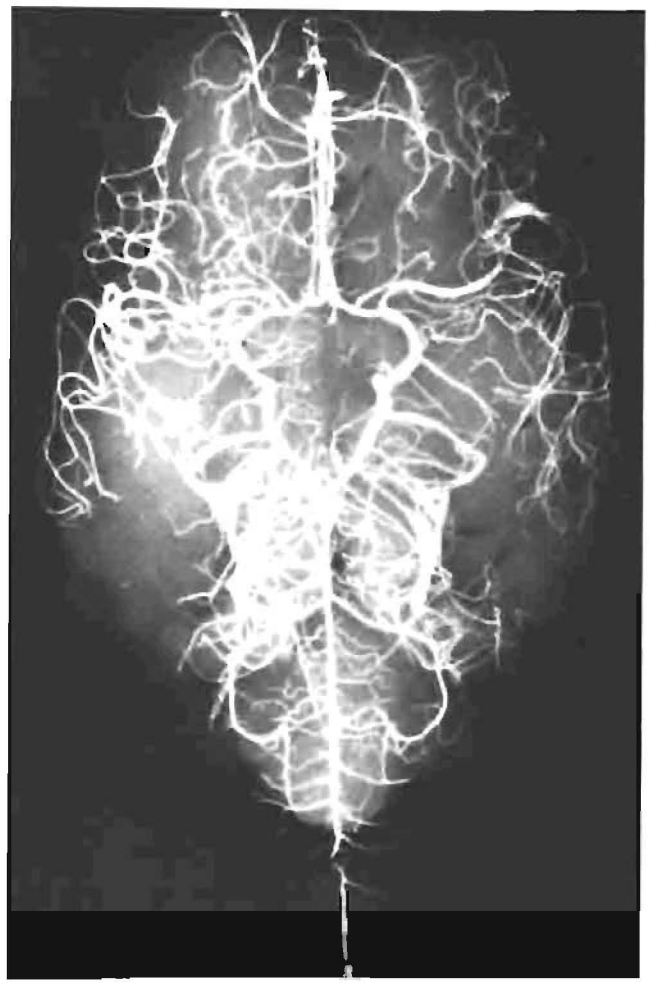

Fig. 5. Brain of the sheep. Vinylite/lead power injection of vessels. Roentgenography.

Fig. 6. Clearing technique of spalteholtz-Poulhès. Nuclear cerebellar arteries of a domestic ruminant.

Fig. 7. Clearing technique of Spalteholtz-Poulhès. Cortex cerebelli arterioles of a domestic ruminant.

dase method for demonstrating haemoglobin in red blood cells within the capillaries. Thus for successful demonstrations of capillaries they must be filled with blood (Figs. 8, 9.). The technique was carried out as follows –

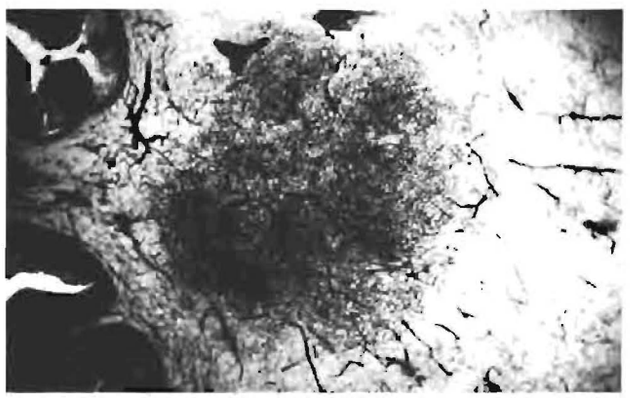

Fig. 8 Pickworth-Fazio technique. Nuclear cerebellar arteries of a domestic ruminant.

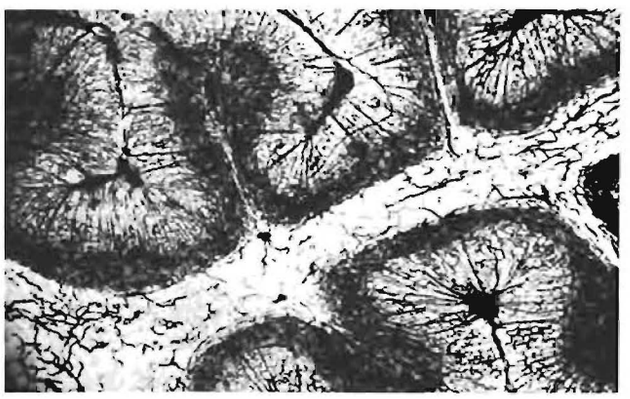

Fig. 9. Pickworth-Fazio technique. Cortex cerebelli arterioles of a domestic ruminant.

1. The fresh material was fixed in formol saline solution (water – 85 mℓ; formalin – 15 mℓ; sodium chloride – 2 g).
2. Sections of 2 cm thickness were cut 24 h later and placed into a saturated solution of salt and sugar with 10 % formalin for 48 h.
3. The sections were washed in running water for 10 s.
4. Frozen sections of 100 μm (0,1–0,3 mm) were cut and washed in distilled water.
5. The frozen sections were placed into a freshly mixed benzidine and nitroprusside reagent for 60 min at 37 °C and then washed for 10 s in distilled water. The benzidine solution was prepared initially exactly according to prescription by dissolving 100 mg of benzidine in 0,5 mℓ glacial acetic acid and adding 20 mℓ distilled water. The sodium nitroprusside solution was prepared by dissolving 80 mg sodium nitroprusside in 80 mℓ distilled water. These two solutions were added to one another immediately before use and agitated frequently. Nevertheless, by saturating an aliquot of glacial acetic acid with benzidine and using 0,5 ml of this solution to add to the prescribed 20 ml of watter, more intensive staining was obtained.
6. The sections were placed in 0,05 % hydrogen peroxide at 37 °C for 60 min with frequent shaking. This dilution was freshly prepared before use.
7. The sections were washed in distilled water, dehydrated in ascending grades of ethyl alcohol and cleared in two or more changes of xylene.
8. The sections were mounted in Canada balsam.

CONCLUSIONS

Differently stained injection masses of gelatine, latex and vinyl resin were found to be the most satisfactory sutbstances for the demonstration of the blood vessels of the brain. A concentration of 6–10 % of gelatine for study of deep vessels and 20–25 % for the superficial ones were established as the optimum. Latex has the advantage of toughness and elasticity and it can be injected into preserved specimens. Vinyl resin produces a rigid case of arteries if the vessels are flushed with acetone prior to the injection.

Vascular beds of excellent quality can be obtained by corrosion of specimens injected with vinyl, resin and latex provided the maceration is done with 10 % of potassium hydroxide solution. Vinyl resin is superior to latex since less shrinkage takes place. Stereo-roentgen-ray examination after injection of vinylite acetone solution to which red lead powder has been added produced the best results.

The clearing technique of Spalteholtz, mofified by Poulhès and the Pickworth-Fazio-technique were chosen as the best methods to demonstrate the microcirculation.

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BOOK REVIEW — BOEKRESENSIE

FINANCING OF HEALTH SERVICES

Report of a WHO Study Group. *World Health Organization Technical Report Series*, 1978, No. 625 (ISBAN 92 4 120625 x).
117 pages, price: Sw.Fr. 11.-. French and Spanish editions in preparation

Many countries are planning to reorient their health services so that they contribute more effectively to economic and social development as a whole. In the developing countries, this means improving services for the poor, especially in rural areas and in slums on the urban fringes. However, the rate at which these improvements are made is severely limited by the shortage of funds. By re-examining all sources of finance for all health-related activities, it should be possible to find ways of speeding up progress.

Unfortunately, information is rarely available about total expenditure on health-related activities – spending by organs of government other than the ministry of health, by social security agencies, and by charitable bodies, and particularly expenditure in the private sector, including that on traditional health care. Only when all sources of finance are brought together, and an analysis is made of what precise services are financed by what funds in particular geographical areas, is it possible to consider what changes can be made in order to move towards the new objectives.

What is therefore needed is to develop a methodology for cl
lecting it. Such information should help in examining how far it is possible to develop new sources of finance, to redeploy funds already devoted to health purposes, or to release resources by more efficient methods of operation for use in areas of high priority.

In November 1977, WHO convened a study group on financing of health services, with participants from all WHO regions, to tackle this problem. The group was asked to examine techniques for obtaining and analysing informaion related to all sources of finance in the health sector and for identifying all sources of health expenditure. It also considered the question, once sufficiently accurate data are available, how best to use the results of financial surveys for improved planing.

Part of the group's work was to examine a number of case studies based on financing surveys carried out in developing countries.

In its report, the group was able to draw important conclusions and make a number of recommendations to countries, research centres, and WHO. One welcome finding was that it is possible to collect and analyse information on financing and expenditure, of sufficient reliability and at modest cost, even for the private sector. Annexes summarizing surveys conducted in Bangladesh, Botswana, the Republic of Korea and Senegal form a substantial part of the report. Further annexes deal with the methodology of a study of health sector financing in Latin America and the role of social security financing in the provision of medical care.

This report, aimed at policy-makers, planners, administrators and health professionals interested in ensuring that scarce resources for health are used effectively and equitably, tackles an important subject that is seldom given the emphasis it deserves.

A PREVIOUSLY UNRECORDED FEEDING SITE ON CATTLE FOR THE IMMATURE STAGES OF THE SPINOSE EAR TICK, *OTOBIUS MEGNINI* (DUGÈS, 1844)

G.M. BULMAN* AND JANE B. WALKER†

ABSTRACT: Bulman G.M.; Walker J.B. **A previously unrecorded feeding site on cattle for the immature stages of the spinose ear tick,** *Otobius megnini* **(Dugès, 1884).** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 107 (En) Ayacucho 2345, Olivos, (1636) Buenos Aires, Argentina.

During the rainy season in the central valley of Cochabamba, Bolivia, the larvae and nymphae of this tick were found feeding under the tails of dairy cattle as well as in their ears. The conditions that apparently favour the temporary infestation of this site on some cows are discussed briefly.

RESUMEN: Bulman G.M.; Walker J.B. **Un sitio de parasitación en vacunos no registrado previamente para los estadios inmaduros de la garrapata espinosa auricular,** *Otobius mengnini* **(Dugès, 1884).** *Journal of the South African Veterinary Association* (1979) **50** No. 2 XXX (En), Ayacucho 2345, Olivos, (1636) Buenos Aires, Argentina.

Durante la estación de lluvias en el valle central de Cochabamba, Bolivia, larvas y ninfas de esta garrapata se encontraron debajo de la cola de ganado lechero, así mismo como en la region auricular. Las condiciones que aparentemente faborecen la infestacion temporaria en este sitio, de algunos vacunos, es discutida brevemente.

INTRODUCTION

The spinose ear tick, *Otobius megnini,* was originally described in 1884 from specimens collected in northern Mexico. It is now known to occur in many of the drier, often hilly parts of North and South America and has also been recorded in Hawaii and India[4 3 8]. Initial introduction into Africa is thought to have occurred with stock imported from America. It was first recognized in the Cape Colony in 1898[1] and has since spread through many of the drier areas of the subcontinent as far north as Zaire, Zambia and Malawi[7 9].

The adults of this tick do not possess functional mouthparts and are free-living. Only the immature stages are parasitic and have been found on most domestic animals (especially ungulates) and sometimes on man. In America they have also been found on various wild animals[8] but in Africa the only wild animal host known so far is, strangely enough a tortoise[9]. Ostriches have also been recorded as hosts[1].

As its common name indicates, the spinose ear tick practically always feeds in the ears of its hosts, though Bedford[1] quotes Storey as having once seen "two almost fully engorded nymphs attached to the bodies of sheep". The larvae usually attach deep in the ear canal, consequently light infestations can easily be overlooked and the use of a curette is recommended to detect them. Even when few ticks are present, infested animals can suffer severely. The tick bites often ulcerate and the ear drums and auricular nerves may become damaged. In heavy infestations the ear canal become completely blocked with reddish-brown masses of wax, ticks and debris, and abrasions, caused when the animals rub or scratch their ears, may attract blowflies. The animals show increasing signs of nervous and digestive disorders, lose condition and, especially in the case of calves, sheep and goats, may die. Rich[8] described a "head-heavy" stance in infested cattle, followed later by muscular incoordination, collapse and death within two weeks. He knew of only one animal showing clinical symptoms that recovered after removal of the ticks.

Howell, Walker & Nevill[5] stated that "the spinose ear tick is not known to transmit any pathogens". Jellison *et al*[6] showed that *O. megnini* can harbour *Coxiella burneti,* the causative agent of Q fever, but Bell *et al*[2] commented that their attempts to transmit this organism to cows via infected spinose ear ticks gave indefinite results.

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OBSERVATIONS AND DISCUSSION

From 1975–1977 the senior author was stationed in Bolivia at Cochabamba, in a climatically dry but well-irrigated valley at 2600 m alitutude. *O. megnini* nymphae were found in the ears of cattle on dairy farms in this area throughout the year. These tick infestations often caused a drop in milk production and during the cold winter months and in spring treatment by means of ear swabs dipped in ixodicide solutions became necessary.

During the short rainy seasons (mean rainfall 340 mm) in two consecutive summers spinose ear ticks were also found under the tails of a number of Holstein dairy cows for periods of up to 60 days. In 1975 these ticks were first discovered under the tails of the cows after the onset of the rains in late October. During 1976/77, however, the main rains did not start until February, though there were a few cloudbursts the previous December followed by a drought, and coincidentally *O. megnini* was not found under the cow's tails until February. During the dry seasons from March to September spinose ear ticks were found only in the cows' ears, never under their tails, and they were never found under the tails of calves.

In the wet season cattle in the Cochabamba dairies are confined to their corrals, where they are also milked and fed. The ticks probably breed in cracks and crevices in the milking sheds. Only cows that habitually slept in these sheds, close to these breeding places, became infested under their tails. Those that bedded down outside, where the ticks probably could not survive on the open ground because of the extremely wet, muddy conditions, were infested in their ears only. Ear infestations may be acquired when the cows are confined in stanchions in the sheds at milking time.

The number of ticks found under the tail varied from the odd specimen to clusters of approximately 150 at the height of the rainy season and included larvae as well as nymphae. The infested area was approximately 2–10 cm above the anus, and on the whole those tails with a well grooved and thus more protected area seemed to be preferred to the more rounded tails. The

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ticks also much preferred cows with dark-coloured skin that felt slightly greasy to the touch under their tails, rather than those with white, rather dry skin in this particular area. The percentage of adult cattle per herd that became infested under their tails seldom reached 15, even at peak periods of infestation. There was no apparent relation between the number of ticks in the ears and those under the tail.

In South Africa it has long been known that spinose ear ticks are most numerous in areas where stock are penned at night, especially when the kraal walls are built of cowdung bricks, or stones and earth, and cannot be disinfected[1]. As yet the authors know of no instances of these ticks attaching under the tails of cattle in South Africa, northwest Argentina or any areas of Bolivia other than that already indicated. The possibility should be noted, however, especially during the treatment of herds to reduce tick populations.

ACKNOWLEDGEMENTS

The authors thank Drs E J Morini and C B de Grillo Torrando (Department of Parasitology, School of Veterinary Medicine, University of Buenos Aires) and Dr H Hoogstraal and Mrs H Y Wassef (U.S. NAMRU-3, Cairo), for comparing their specimens with others from South America, and confirming their original identification.

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BOOK REVIEW — BOEKRESENSIE

POVERTY, DEVELOPMENT AND HEALTH POLICY

. ABEL-SMITH, with A. LEISERSON
Geneva, World Health Organization, 1978 (Public Health Papers No. 69) ISBN 92 4 130069 8. 108 pages.
Price: Sw.Fr. 10.-, US$ 5.-. French and Spanish editions in preparation.

The improvement of health is an essential element of national development, and this book describes some of the ways in which work in the health sector can support the national planing of rural development aimed at the relief of poverty. In the first part, which is concerned with the interdependence of health policy and national planning, the book treats poverty as one of the main causes of low health standards and shows how health administrators can help to improve the standard of living. It is not enough for them to fulfil the conventional role of formulating health plans and obtaining funds to carry them out: they must participate in development in its widest sense. The authors argue that the attempt to link the provision of health services with increases in industrial productivity is often unhelpful, and that it is better to adopt wider, non-economic criteria. Health services might be provided, for example, as a way of redistributing wealth and as a move to greater equity, even if they do not have any lasting effect on health and no effect on production. They might then contribute to social cohesion and give a sense of psychological security.

Although it is not intended to be a textbook on health economics or planing, the book does include a brief explanation of the economic framework used in national planning in order to indicate the kind of knowledge the health administrator will need.

The second part of the book deals with the economics of health services, since economic analysis can be used to find ways of achieving health objectives with less use of resources. Thus several chapters are devoted to the analysis of health service expanditure, the financing of health services, and cost-benefit and cost-effectiveness analysis, emphasis being given to detailing the economic benefits of health programmes.

The final chapter, on low-cost services, stresses the need to involve the local population in the provision of services. The most important parts of health care relate to the basic aspects of life – an adequate diet, safe water supplies, safe disposal of excreta, personal hygiene, and the control of vectors and rodents – and in all these areas the community can do much for itself. Moreover, traditional medical practitioners and midwives are still widely consulted in many developing countries and could usefully be brought within the health care system, with appropriate training and assistance.

The book is addressed primarily to health administrators and to teachers of health personnel in developing countries and the authors have presented their material with a minimum use of economic terminology.

SIMPTOME VAN HONDSDOLHEID BY HUIS- EN PLAASDIERE IN SUID-AFRIKA EN SUIDWES-AFRIKA

B.J.H. BARNARD

ABSTRACT: Barnard B.J.H. **Symptoms of rabies in pets and domestic animals in South Africa and South West Africa.** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 109 (Afr/En) Section Virology, Vet. Res. Inst. 0110 Onderstepoort, Rep. of South Africa.

The most obvious symptoms of rabies in farm animals and pets in South Africa and South-West Africa are discussed in the light of information obtained during routine examination of specimens for the 10-year period 1967–1976.

More than 55 % of the cases encountered were cattle in which the most obvious symptoms were salivation (92 %), bellowing (69 %), aggressiveness (47%), paresis or paralysis (30 %) and straining (12 %). Unlike cattle, the most obvious symptom in goats was aggressiveness (83 %). Salivation was observed in only 29 % of goats but, like bellowing in cattle, bleating was very obvious in 72 % of cases. Sheep were usually quiet, but 67 % were aggressive. Salivation was observed in 30 %, while 27 % showed an abnormal sexual desire.

The second highest incidence of rabies was recorded in dogs (20 %). Aggressiveness was the most obvious symptom (71 %) followed by salivation (48 %), paresis and paralysis (28 %) and barking (11 %). With the exception of salivation and paresis, which were rarely encountered, aggressiveness was the only symptom observed in cats. Several cats were encountered with rabies-like symptoms due to organic phosphate poisoning. The most obvious symptoms in horses and donkeys were aggressiveness (77 %), paresis or paralysis 33 %), the chewing of foreign matter (33 %) and salivation 22 %).

It is obvious that other conditions can easily be confused with rabies. Therefore every possible cause for rabies-like behaviour must be considered and eliminated to avoid unnecessary destruction of animals.

INLEIDING

Die simptome van hondsdolheid word gewoonlik in 'n prodromale stadium, 'n stadium van opgewondenheid en 'n verlammingstadium ingedeel. Die tydsduur van die drie stadiums variëer van dier tot dier en van spesie tot spesie. Waar opgewondenheid oorheers, word na malhondsdolheid (furious rabies) verwys terwyl die term domhondsdolheid (dumb rabies) gebruik word waar verlamming oorheers.

Die "diaboliese" aard van die hondsdolheidvirus veroorsaak dikwels dat die virus sy slagoffers so aantas dat hul aggresiwiteit verhoog en daardeur bytwonde toegedien word. Dit verseker die voortbestaan van die virus. Langdurige waarneming en 'n deurtastende ondersoek van 'n dier met verdagte hondsdolheid is dus nie wenslik nie en kan selfs lewensgevaarlik wees. Aangesien hondsdolheid 'n aangeebare en dodelike siekte is moet 'n kliniese diagnose deur 'n laboratoriumondersoek bevestig word: so 'n dier moet dus van kant gemaak word. Hondsdolheid kan egter geredelik met ander bepaalde en onbepaalde siektetoestande van plaas- en huisdiere verwar word, gevolglik word die veearts dikwels voor 'n moeilike besluitneming geplaas en wil hy 'n dier nie onnodig laat afmaak nie.

Gegewens oor die simptome en ander samehangende inligting oor hondsdolheid by huis- en plaasdiere word in hierdie stuk saamgevat.

MATERIAAL EN METODE

Alle bevestigde hondsdolheid gevalle by huis- en plaasdiere in Suid-Afrika en Suidwes-Afrika gedurende die tydperk 1967 tot 1976 is in berekening gebring. Die verskillende spesies word afsonderlik behandel en sluit 456 beste, 31 skape, 18 bokke, 9 perde en donkies, 194 honde en 86 katte in.

Gegewens oor simptome, inkubasietyd en die duur van die siekte vandat abnormale gedrag of ander simptome waargeneem was totdat die dier gevrek het, is verkry van die insenders van materiaal ter bevestiging van kliniese diagnoses en word in tabelle 1 tot 3 saamgevat. Gevalle waar die beskikbare gegewens onvoldoende was is by die berekening van persentasies buite rekening gelaat.

RESULTATE

Simptome

Tabel 1: SIMPTOME WAARGENEEM BY HUIS- EN PLAASDIERE MET HONDSDOLHEID IN SUID-AFRIKA EN SUIDWES-AFRIKA GEDURENDE DIE TYDPERK 1967 TOT 1976

	Bees	Skaap	Bok	Perd en donkie	Hond	Kat
Aantal gevalle	456	31	18	9	194	86
Simptome beskryf by	408	31	18	9	100	80
Simptoom						
Aggressiwiteit	47*	67	83	77	71	74
Salivasie	92	30	29	22	48	3
Parese tot verlamming	30	16	17	33	28	4
Bulk, blêr, tjank of blaf	69	3	72	–	11	–
Hardloop rond	3	–	6	0	6	0
Verhoogde seksdrang	4	27	–	–	–	–
Parsing	12	–	–	–	–	–
Diaree	1	–	–	–	2	–
Hidrofobie	3	–	–	–	5	–
Skynbaar blind	1	–	–	–	–	–
Net lusteloos	1	–	–	–	8	–
Spiertrekkings	1	–	–	–	3	–
Makker as normaal	2	–	–	–	–	–
Kners op tande	–	6	6	–	–	–
Byt vreemde voorwerpe	–	–	–	33	–	–
Onderkaak verlamming	–	–	–	–	13	–
Loop in sirkels	–	–	–	22	4	4
Knip oë	–	–	–	–	2	–
Kruip weg	–	–	–	–	1	–
Verdwyn vir tyd	–	–	–	–	–	7

*Uitgedruk as 'n % van die diere waarvan die simptome beskryf is

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Inkubasietyd

Tabel 2: INKUBASIETYD VAN HONDSDOLHEID IN BEESTE, SKAPE EN HONDE IN SUID-AFRIKA EN SUIDWES-AFRIKA GEDURENDE DIE TYDPERK 1967 TOT 1976

Tyd in dae	Bees	Skaap	Hond
10 – 15	8	4	4
16 – 20	9	10	1
21 – 30	12	7	8
31 – 40	6	–	–
41 – 60	3	–	–
61 en langer	4	–	–

Duur van die siekte

Tabel 3: DIE DUUR VAN HONDSDOLHEID IN BEESTE EN HONDE IN SUID-AFRIKA EN SUIDWES-AFRIKA GEDURENDE DIE TYDPERK 1967 TOT 1976

Tyd in dae	Beeste	Honde
2 en minder	14	3
3	18	2
4	10	3
5	9	3
6	2	1
7	3	1
8	–	1
9	–	1
10	3	1
14	–	1

BESPREKING

Honde

Wanneer mens betrokke is by hondsdolheiddiagnostiek is dit treffend dat 'n groot aantal diere, veral honde en katte, onnodig doodgemaak word om 'n diagnose wat op grond van simptome gemaak is, te bevestig. Dit is ook opvallend dat min simptome en dikwels net die toediening van bytwonde in sulke gevalle beskryf word.

Hierdie optrede kan heelwaarskynlik toegeskryf word aan vrees vir die siekte en die emosionele betrokkenheid van die betrokke persone. Indien in ag geneem word dat honde en katte hulle normaalweg verdedig deur te byt of te krap en moeite gedoen word om die moontlike oorsake vir so 'n optrede na te spoor, kan die onnodige vankantmaak van honde en katte verminder word. Volgens die resultate (Tabel 1) is aggressiwiteit die mees algemene simptoom by honde maar daar is meesal ook ander duidelik waarneembare simptome teenwoordig soos salivasie, parese, verlamming, ongemotiveerde tjank- en blafgeluide, of 'n verlamming van die onderkaak.

Omdat die inligting waaruit hierdie stuk saamgestel is, slegs die opvallendste simptome beklemtoon, kom die feit dat 'n dier met hondsdolheid siek is, nie duidelik uit nie. Verlies van eetlus en 'n algemene siek indruk onderskei 'n hond met hondsdolheid van 'n bevoeterde hond. Die duur van die siekte (Tabel 3) kan ook 'n aanduiding gee of ander toestande betrokke is. By die meeste honde het die siekte twee tot vyf dae geduur. Die geval wat 14 dae lank siek was, was 'n teef wat geaborteer het. Daarna het sy geleidelik aggressiewer geword en op die 14de dag gevrek. Dit kan as 'n uitsonderlike geval beskou word. Dit is opmerklik dat ander bekende simptome van hondsdolheid by honde, soos die vreet van vreemde materiaal en die lek van die plek waar besmetting plaasgevind het, so min waargeneem is. Hidrofobie is ook relatief min waargeneem maar het saam met verlamming en braking laat gedurende die siekte ontwikkel en word derhalwe selde gesien omdat diere dikwels voor die stadium vankant gemaak word.

Katte

Die uitstaande simptoom by katte, naamlik aggressiwiteit, is by 74 % van die diere (Tabel 1) waargeneem. Dit het dikwels tot die toediening van bytwonde gelei. Katte was oor die algemeen aggressiewer as honde en uiters gevaarlik. Slagoffers is dikwels van agter aangeval, aan die gesig gebyt, en die katte moes soms bo-op hul slagoffers van kant gemaak word of van hulle los geskeur word.

'n Onbekende siektetoestand van katte wat deur 'n geweldige aggressiewe optrede soortgelyk aan dié van hondsdolheid gekenmerk word is telkens teëgekom. Sulke gevalle was nie tot 'n sekere gebied beperk nie maar kom skynbaar meer gedurende somermaande voor. In die fluoresserende teëliggaampie-toets vir hondsdolheid word veelvuldige wit tot ligblou fluoresserende struktuurtjies in die breinweefsel van sulke katte waargeneem. Hierdie struktuurtjies lyk na niespecifiek-fluoresserende kokki, maar kan nie met 'n histopatologiese ondersoek waargeneem word nie. Oorspuiting van breinweefsels van sulke katte na ander katte of muise het nog geen positiewe resultaat opgelewer nie en kweking of bloedagarplate was altyd negatief.

Gevalle met hondsdolheidagtige simptome het ook voorgekom by katte wat met Pulvex*, 'n wasmiddel vir honde, behandel was.

Beeste

Die siekte in beeste is gekenmerk deur salivasie (92 %), bulk (69 %), aggressiwiteit (47 %) en parese tot verlamming (30 %)(Tabel 1). Parsing het by 12 % voorgekom en saam met die ander simptome kan dit as van diagnostiese waarde beskou word. Ofskoon 'n hoë persentasie beeste bogenoemde simptome en dikwels 'n kombinasie van drie of meer daarvan getoon het, kan dit nie as kenmerkend van hondsdolheid alleen beskou word nie. In talle gevalle met "tipiese" simptome van hondsdolheid kon die diagnose nie bevestig word nie, met die uitsondering van dié gevalle waar parsing waargeneem was en wat net by bevestigde gevalle voorgekom het. Tot dusver is slegs een geval in Suid-Afrika bekend waar 'n persoon hondsdolheid opgedoen het nadat hy deur 'n bees gebyt is, terwyl talle gevalle van hantering van dol beeste bekend is. Dit dui dus daarop dat die versigtige hantering van 'n bees met hondsdolheid nie 'n groot risiko inhou nie. Wanneer verdagte simptome by beeste en veral in hondsdolheid-vry gebiede voorkom, behoort spesiale moeite gedoen te word om ander oorsake vas te stel.

Die inkubasie tyd by beeste het vanaf 10 tot meer as 60 dae gevariëer maar 75 % van die gevalle het binne 30 dae na besmetting siek geword (Tabel 2). By die

*'n Organiese fosfaat – Chlorpyrifos (Dursham) saamgestel deur Coopers, Posbus 679, Kemptonpark 1620.

meeste gevalle met 'n lang inkubasieperiode kon die moontlikheid van herbesmetting na die blootstelling nie uitgeskakel word nie. Een van die gevalle wat langer as 60 dae na besmetting siek geword het, was die kalf van 'n koei wat drie dae na kalwing dood is aan hondsdolheid. Die kalf wat onder observasie gehou is en dus nie weer besmet kon geraak het nie, het 72 dae later siek geword. In hierdie geval het oordraging volgens waarneming nie deur 'n bytwond geskied nie, maar waarskynlik deur kontak en kan dit die rede wees vir die lang inkubasieperiode.

Skape en Bokke

Alhoewel 'n verhoogde seksdrang net by 27 % van bevestigde gevalle in skape waargeneem is, was dit die kenmerkendste simptoom. Dié skape het op mekaar gery, aander ooie se lammers afgeneem en dikwels geurineer. Selfs skape wat nie lammers gehad het nie, het so reageer. Sewe-en-dertig persent was aggressief. Dit het tot uiting gekom deurdat hulle ander skape, vreemde voorwerpe soos mure en pale, en mense stormgeloop en gestamp het. Salivasie het by 30 % voorgekom en parese tot verlamming by 16 %. Slegs een skaap het aanhoudend geblêr. Hierteenoor het bokke baie soos beeste gereageer; 72 %het geblêr en 83 %het aggressief opgetree deur ander diere en mense rond te jaag en te stamp, terwyl 17 % ook gebyt het. Salivasie het by 17 % voorgekom teenoor 30 % by skape en 92 % by beeste.

Perde en Donkies

Slegs nege gevalle van hondsdolheid in perde en donkies is bevestig en aggressiwiteit was die uistaande kenmerk. Aangetaste perde en donkies het aanvallend opgetree. Sewe van die nege het mense of ander perde gebyt, twee het ook vreemde voorwerpe soos pale en drade gebyt, terwyl drie hulself gebyt het. Dit is nie bekend of die perde wat hulself gebyt het dit op die oorsponklike bytwonde gedoen het nie. Twee perde het in sirkels geloop terwyl salivasie by slegs twee waargeneem was.

GEVOLGTREKKING

Aggressiwiteit was die één simptoom wat by 'n hoë persentasie van al die spesies waargeneem is. Salivasie, parese en verlamming is by al die spesies met die uitsondering van katte waargeneem. Salivasie was veral by beeste 'n uitstaande simptoom. Vermeerdering van normale geluide soos bulk en blêr is hoofsaaklik by beeste en bokke waargeneem. Kenmerkende, maar nie dikwels waarneembare simptome nie, was parsing by beeste, verhoogde seksdrang by skape en onderkaakverlamming by honde. Nieteenstaande die redelik kenmerkende simptome, het daar tog enkele atipiese gevalle voogekom soos diere wat net lusteloos was of net 'n parese getoon het. Gevolglik behoort enige persoon wat met diere te doen het, veral in hondsdolheidgebiede, altyd die moontlikheid van hondsdolheid te oorweeg en dienooreenkomstig op te tree.

BOOK REVIEW — BOEKRESENSIE

AIR QUALITY IN SELECTED URBAN AREAS 1975–1976

Published under the joint sponsorship of the United Nations Environment Programme and the World Health Organization, Geneva, World Health Organization, 1978 (*WHO Offset Publication* No. 41: ISBN 92 4 170041 6) 42 pages Price: Sw.Fr. 9.-.

This volume, the second report to emerge from WHO's air quality monitoring project, presents data on sulfur dioxide and suspended particulate matter or smoke collected in some 125 stations that monitored urban air quality in 26 countries or areas in 1975–1976. Data for 1973 and 1974 appeared in an earlier WHO publication[1] which also gives further information on the project's objectives, the selection of monitoring sites, and data processing procedures.

The data are tabulated as cumulative frequency distributions for each sampling site, by city. Information on sampling site location, number of samples analysed, minimum and maximum concentrations, and arithmetic and geometric means is also shown. The analytical method used and the averaging period are indicated at the top of each table. Measurement methods are summarized in an introductory section.

Details of the WHO collaborating centres and national centres that reported data in 1975–1976 appear in an annex. A second annex presents a report on an inter-laboratory quality control study carried out in 1975 to compare the results obtained in sulfur dioxide analyses. The study, in which 10 laboratories took part, was restricted to an evaluation of analytical accuracy; sampling competence was not tested.

The WHO air quality monitoring project, which began operations in 1973, is a collaborative undertaking in which Member States contribute data from a number of urban air monitoring stations. One of its purposes is to provide an input for the Global Environmental Monitoring System (GEMS). In 1975, with the support of the United Nations Environment Programme (UNEP), it substantially expanded its operations. By March 1978, 43 countries or areas, and some 170 monitoring stations were taking part in the project, which is now being extended to include data on nitrogen dioxide and lead (in particulate matter) collected from traffiic-related stations.

Air pollution remains a crucial problem in most large cities today. The continuity of the data reported, coupled with the project's steadily increasing scope, suggest that this new volume, like its predecessor, will prove avaluable tool both to public health and other national and municipal authorities and to planners wishing to study pollution levels and trends.

[1] *Air quality in selected urban areas, 1973–1974*, Geneva, World Health Organization, 1976 (WHO Offset Publication No. 30).

THE RESTRAINT OF THE CAPE HUNTING DOG *LYCAON PICTUS* WITH PHENCYCLIDINE HYDROCHLORIDE AND KETAMINE HYDROCHLORIDE

H. EBEDES and M. GROBLER

ABSTRACT: Ebedes H.; Grobler M. **The restraint of the Cape Hunting Dog** Lycaon Pictus **with Phencyclidine Hydrochloride and Ketamine Hydrochloride**. *Journal of the South African Veterinary Association* (1979) **50** No. 2, 113 (En) Private Bag X5020, Stellenbosch 7600 Rep. of South Africa.

Phencyclidine HCl and ketamine HCl were used as immobilizing agents on six captive Cape Hunting dogs. Both drugs were suitable and three dogs immobilized with ketamine HCl at a dosage rate of 7 mg/kg had smoother and shorter recovery periods than the three animals immobilized with phencyclidine HCl.

INTRODUCTION

Phencyclidine HCl (Sernylan Parenteral, Parke-Davis) has been used as an immobilizing and anaesthetic agent for a wide spectrum of captive and free-ranging African carnivorous animals[1 3 4 5 6 9 10 11 12]. Smuts *et al*[14] recorded the use of ketamine HCl (Parke-Davis) for the field immobilization of large wild felines such as lion *(Panthera leo),* leopard *(Panthera pardus)* and cheetah *(Acinonyx jubatus).* In a later publication, Smuts[13] reported favourably on the immobilization and restraint of spotted hyaena *(Crocuta crocuta)* using a combination of ketamine and azaperone.

Phencyclidine HCl was probably first used by Kroll[10] to immobilize a captive Cape Hunting dog *(Lycaon pictus).* The animal was immobilized in 5½ minutes with a dose of 1 mg/kg. Seal *et al*[12] immobilized three Cape Hunting dogs with 0,8–1,0 mg phencyclidine HCl. Although ketamine HCl has been used extensively for the anaesthesia of domestic dogs[2 7 8] its use on the Cape Hunting dog has not been recorded previously. This paper reports on the use of phencyclidine HCl and ketamine HCl in six captive Cape Hunting dogs in the Etosha National Park.

MATERIALS AND METHODS

The dogs were kept in captivity from the age of approximately one month to 10 months. Because they were destined to be released in the wild it was necessary to restrain them for translocation, marking with ear-tags, weighing and innoculations against rabies, canine distemper, canine hepatitis and leptospirosis*.

The Palmer Cap-Chur gun and one ml and three ml projectile darts were used for darting the captive dogs from distances of three to five metres. The darts were fired with green (low charge) ramsets. The darts were directed mainly to the muscles of the hindquarters. Phencyclidine hydrochloride, 20 mg/ml (Sernylan Parenteral, Parke-Davis) and ketamine hydrochloride, 50 mg/ml (Ketalar, Parke-Davis) were the drugs used to achieve immobilization.

RESULTS

The reslts of the immobilizations are given in Table 1. Dog No. 1 received the largest dose and was anaethetised in 4½ minutes. Dog No. 2 took a long time to become immobilized and the reason for this was that he bled from the dart wound and a portion of the phencyclidine HCl was not absorbed into the bloodstream.

The 3 animals receiving 150 mg ketamine HCl (ap-

Table 1: PHENCYCLIDINE HCl AND KETAMINE HCl FOR RESTRAINING CAPE HUNTING DOG

No.	Sex	Weight kg	Phencyclidine mg	Ketamine mg	Imm. Time	Temp. °C	Pulse per min.	Resp. per min.	Recovery Time
1.	M	25	30 1,2 mg/kg	–	4 min 30 sec	39	62	26	± 4 hours
2.	M	25	20 0,8 mg/kg	–	23 min	40,3	108	42	± 2 hours
3.	M	24	20 0,83 mg/kg	–	8 min 46 sec	39,4	74	28	2½ hours
4.	M	21,8	–	150 7 mg/kg	4 min 10 sec	39,5	118	26	1 hour 40 min.
5.	F	21,8	–	150 7 mg/kg	5 min 45 sec	39	90	52	45 min.
6.	F	22,7	–	150 7 mg/kg	8 min 25 sec	40,5	92	32	50 min.

*Vaccines: (a) Enduracell d-h, (canine distemper hepatitis vaccine – Norden Laboratories); (b) Lepto c-i, (leptospira canicola – ictero haemorrhagia bacterin – Norden Laboratories); (c) Endurall-R, (Rabies vaccine – Norden Laboratories).

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proximately 7 mg/kg) were satisfactorily anaethetised, recovered more smoothly and were on their feet sooner than the three animals injected with phencyclidine HCl. Mean immobilization time for phencyclidine was 12 minutes 5 seconds (n = 3); mean recovery time was two hours 50 minutes. Mean immobilization time for ketamine HCl was six minutes seven seconds (n = 3) and mean recovery time was one hour and five minutes.

Salivation occurred in all the animals. Epileptiform convulsions lasting 10–15 seconds occurred in dog No. 1 and these were as a result of the high dose of phencyclidine HCl. The higher dose also caused a longer recovery time. No pain was apparent in any of the animals during the insertion of the ear-tags and the injection of the vaccines which indicated satisfactory analgesia.

DISCUSSION

A comparison between the two drugs used for immobilizing Cape Hunting dogs is not justified because of the small numbers in the sample. Ketamine HCl however had a shorter recovery period than phencyclidine and is a useful drug for short immobilization procedures in Cape Hunting dogs.

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CONGRESS NEWS — KONGRESNUUS

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The 16th Congress of the International Association of Biological Standardization is to be held in San Antonio, Texas, U.S.A., September 16–20, 1979. The theme is: Animals: Their Standardization to Improve Research, Production, and Testing of biologicals. Interested participants should provide appropriate title to either:

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Preliminary program is listed in *IABS Newsletter* No. 34 – December, 1978, or contact one of the above. Registration fee: 100 SwFr (approximately $55,00 U.S.).

SOME ASPECTS OF FEEDING OF BROOD GILTS AND SOWS*

R.O. DE WILDE

ABSTRACT: De Wilde R.O. **Some aspects of feeding of brood gilts and sows.** *Journal of the South African veterinary Association* (1979) **50** No. 2, 115 (En) Laboratorium voor Diervoedingsleer, Fakulteit Diergeneeskunde, Gent, België, Heidestraat 19, B 9220 Merelbeke.

The amounts of feed required during each of the reproductive phases are graphically illustrated and supported by specific recommendations where possible with discussion of the underlying rationale and of the often conflicting considerations. For flushing, an increase of 50 to 100 % of energy requirements above maintenance level is recommended, supplied, for example, by one to two kg maize extra per day during the week before expected oestrus, in order to increase the ovulation rate. During pregnancy a constant feeding level is proposed 2,2 to 2,5 kg of feed with 8,8 MJ of nett energy/kg and 12 % digestible crude protein being necessary. Two days before farrowing the feeding level must be lowered and steps taken to keep the gut contents to a minimum. During lactation the feed has to contain 8,8 MJ nett energy/kg and 14 % digestible crude protein and the intake reduced by 10 to 20 % in the case of early weaning. At weaning a fasting regime is suggested to effect physiological stress.

The relation between feeding level during pregnancy and the mass gain of the sow and number of piglets born, as well as on the birth mass of the piglets, and the relation between N intake and N retention and litter mass at birth are shown graphically.

INTRODUCTION

The main objective of feeding of brood sows and gilts is to enhance fertility. Whereas in fattening pigs the feed efficiency is given by the ratio of feed consumed to mass gain, in breeding animals the feed efficiency can be expressed as the ratio of feed consumed by the sow and her litter between fertilisation and weaning to the total mass of healthy piglets at weaning. (In the case of weaning at three to four weeks, the amount of feed consumed by the piglets is negligible.)

These indices concern optimal feed efficiency. Nevertheless, economic efficiency is as important as nutritional efficiency; often these do not run parallel to one another. Economically it is more important to produce a lesser number of pig carcasses of a significantly higher grade: it often may be preferable to have more but somewhat lighter piglets than a smaller number of heavier piglets.

In this article the practical aspects of feeding breeding sows and gilts in order to maximise economic profits are emphasized.

FEED REQUIREMENTS DURING BREEDING

The optimal requirements for energy, protein, minerals and vitamins have been compiled from literature data by the English Agricultural Research Council[1], whereas a similar compilation by the American National Research Council[11] concentrates more on the minimal requirements. The former review and some more recent data have been used as a guide for the graphical representation (Fig. 1.) of a feeding schedule for female animals during the following periods of reproduction: (a) flushing; (b) pregnancy; (c) farrowing; (d) lactation; (e) weaning.

Flushing

"Flushing" is defined as supplying increased amounts of energy before and at oestrus in order to increase the ovulation rate. An increase of 50 to 100 % of the maintenance requirement can result in the release of two to three additional ova. It can be effected by one to two kg maize or manioc (cassave) extra per day during the week preceding the presumable heat period in nulliparous gilts and between weaning and oestrus in older sows. It is not necessary to feed a more expensive protein-rich compound.

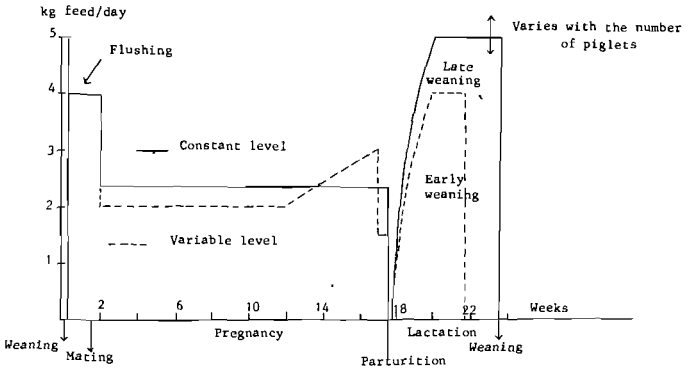

Fig. 1. Feeding scheme for female pigs in pregnancy and lactation.

Some factors must be taken into consideration to obtain this effect.

(a) Primiparous animals respond most favourably[2].
(b) Multiparous sows, which normally have a sufficiently high ovulation rate, respond less favourably, unless they were undernourished during the previous lactation period.
(c) Nulliparous gilts often release an increased number of ova as result of flushing but this is not necessarily followed by an increase in the number of piglets born[8].
(d) Very fat animals, e.g. after a short lactation, or maiden gilts, are less responsive than leaner ones.
(e) A higher ovulation rate may not result in an increased number of piglets born at all. Embryonic mortality during the first weeks of pregnancy may completely offset the advantage gained by a higher ovulation rate. Again the feeding level is important, but now in a reverse way. Indeed, a high feed level maintained for several days and sometimes weeks after mating can increase embryonic mortality[12]. Reduction of the amount of feed to normal levels after each mating is recommended, followed by flushing again, be it only for a few days, as soon as the first signs of an approaching oestrus are visible, in those cases of a preceding unsuccessful

*Based on a lecture given on April 4, 1977, in the Department of Zootechnology, Faculty of Veterinary Science, Onderstepoort, South Africa.

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mating. Even in this short period of two to three days the increased energy supply may be effective[19].

Pregnancy

The requirements during pregnancy are low. For the first and second third of gestation the needs equal those of maintenance. In the last third there is an inrease of heat production[16], coupled with, and perhaps caused by, an intensive growth rate of the foetuses and the development of the mammary glands. Nevertheless, Wrathall[18] prescribes that the increase should not exceed 10 %. Consequently in practice a constant feeding level is proposed for the whole gestational period. This amounts to 2,2 to 2,5 kg/d of a feed with 8,8 MJ/kg nett energy and 12 % digestible crude protein. This protein level may be lowered, as long as the lysine and the methionine-cystine content does not fall below 0,56 and 0,48 % respectively. The energy requirements are low and this allows one to give a combined ration of more bulky farm products and a protein concentrate.

The concentrate must contain the appropriate amounts of vitamins and minerals according to ARC[1] and NRC[11] standards. There is no specific need for animal protein during pregnancy. If animal protein is replaced by vegetable protein, care must be taken that not only the concentrations of amino acids are correct but also that the ration is supplemented by those vitamins and micro-elements which are present in lesser amounts in the vegetable sources, to wit vitamins A, E, B_{12} and selenium.

The proposed amounts of feed vary between 2,2 and 2,5 kg. This variation of more than 10 % makes allowance for the following considerations:

(a) heavier sows need more than lighter ones;
(b) very lean sows should receive the highest amount;
(c) sows in paddocks require more feed than confined sows;
(d) the degree of worm infestation greatly influences nutritional requirements. In Europe each sow is dewormed at every farrowing. It is more appropriate to deworm the herd rather than each sow individually: all animals are dewormed simultaneously, regardless of their physiological state.

Parturition

Two days before farrowing the feeding level must be lowered. It is important to keep the intestinal contents as low as possible, or at least to provide for regular evacuation of faeces. Round about parturition there is often a stasis of the intestines, resulting in over-filling and abnormal fermentation; proliferation of toxigenic bacteria may occur in the colon. Such a situation is conducive to development of the metritis-mastitis-agalactia syndrome. Good feeding practice can be helpful in preventing this synrome. In herds with a history of high incidence of this disease, part of the ration should be replaced by more bulky feed stuffs, such as wheat bran. This achieves better evacuation of faeces and a decreased fermentation rate in the gut. Sows on an exclusively all-mash ration are rather reluctant to eat wheat bran, so that the incorportion of bran into the ration rather has a more indirect effect by reducing feed intake. Finally, a laxative drug may be given: 10 g $MgSO_4$ daily for three days before parturition brings about a significantly increased elimination of faeces.

Only the nutritional measures are mentioned here. Other factors, such as high temperature, are also considered to be responsible for the agalactia syndrome[9].

Lactation

Fig. 1. clearly illustrates the higher feeding level required during lactation compared to that needed during pregnancy. Milk production varies with the parity number, the stage of lactation and the number of piglets. Some figures of milk yield are given in Table 1. They concern milk production of Belgian Landrace pigs, which are known to be less productive because of the meaty constitution of this breed. The milk composition of these sows amounts to 6 % fat, 5 % protein and 5 % lactose. Converted to cow's milk the production figures on a dry basis have to be doubled. This indicates the high requirements of protein and energy in lactating sows.

The feed allowances are calculated as follows:
(a) for primiparous animals: 2,0 kg + 1 kg/3 piglets;
(b) for multiparous animals: 2,5 kg + 1 kg/3 piglets.
The feed must provide 8,8 MJ energy/kg and contain 14 % digestible crude protein.

Table 1: AVERAGE DAILY MILK PRODUCTION OF BELGIAN LANDRACE SOWS (Kg)[15]

Week of lactation	1	2,0	3	4
First parity	2,0	4,0	5,0	6,0 (Mean of 7,5 piglets)
Second parity	2,5	5,0	6,5	7,0 (Mean of 8,3 piglets)
Third parity	2,75	5,0	6,5	7,25 (Mean of 9,0 piglets)

Bulky feedstuffs are not recommended because of their low energy content. The energy requirements are too high and on such feeds the sow's capacity for ingestion becomes a limiting factor. In our Belgian Landrace pigs we observed a maximum feed intake capacity of about 5 kg/d.

A gradual adaptation to the increased demands for food during lactation should be applied, in keeping with the increase of milk production. Some mass loss of the sow during the first weeks of lactation is unavoidable but there is a general consensus that a nett gain of about 10–15 kg between two succeeding reproductive cycles should be realised, especially in the case of gilts.

The nutritional status of the sow at the end of lactation is important for the subsequent reproductive cycle. Both too fat and too lean sows are prone to reproductive disturbances. Hence the proposed amounts of feed should be lowered by 10–20 % in the case of early weaning (three to four weeks), because the amount and duration of milk production is less. Otherwise it would be difficult to obtain a good flushing effect in lean sows fed on too high a level between weaning and oestrus.

Weaning period

Opinions on feeding during this period are controversial. In Europe it is generally recommended to give no feed at weaning and only a limited amount of water. This practice is followed more to stress the sow than to terminate milk production[10]. It is assumed that any change in housing and management just after weaning

favours increased FSH production. Other such stimuli are:

(a) removing the sow from the piglets, but not the reverse: in this way the piglets are stressed less than the sow;
(b) placing the sow near other sows in the same physiological state just after weaning. Placing them together is disadvantageous because fighting and subsequent trauma may delay the onset of oestrus[7];
(c) changing the feeding pattern as far as both quantity and composition are concerned.

On the other hand, a fasting perod of 24 h after weaning has been considered to reduce the number of piglets in the subsequent litter[13], a finding which could not be confirmed[3 4].

In general, the conflicting results of investigations concerning the feeding of sows at weaning could possibly be explained by differences in the intensity of the stimulus applied by feeding treatment. For the time being one may recommend that the fasting regime be followed, possibly more on the basis of applying an external stressor to elicit oestrus, rather than on the basis of purely nutritional effects.

THE INFLUENCE OF DIFFERENT FEEDING LEVELS UPON REPRODUCTIVE PERFORMANCE

The proposed quantities of feed are applicable to the average sow under European conditions. Allowance must be made for difference in genetic make-up and in climatic and housing conditions. It must be borne in mind that the maternal system can buffer temporary feeding imbalances. Some graphs are given to illustrate the influence of feeding on reproductive performance.

Fig. 2. indicates that any gain in mass, caused by an increase in feeding level during pregnancy, does not necessarily result in a greater number of piglets at birth. There is an optimum at 2,0 to 2,5 kg feed per animal per day. Above this quantity the number of piglets diminish but the mass gain of the sow continues, owing to increased fat deposition[14].

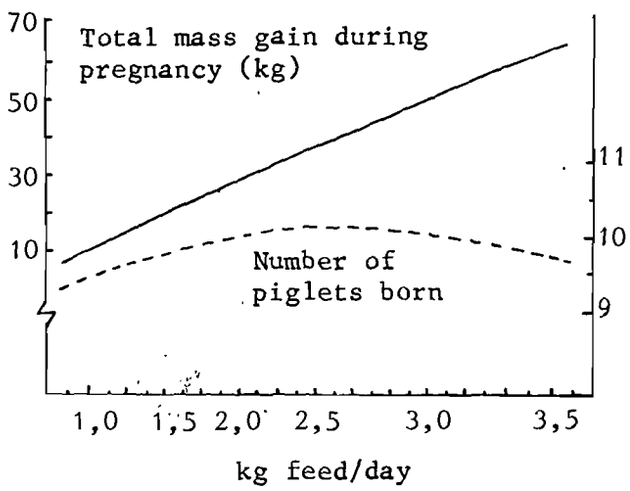

Fig. 2. Relation between the feeding level during pregnancy and the mass gain of the sow or the number of born piglets[14].

Fig. 3. illustrates the effect of protein level on nitrogen retention on the one hand and the litter mass on the other. A decreased protein intake results in a reduced N retention but this reduction is less than the decrease in protein intake. There is some sparing effect which is reflected in a nearly unchanged litter mass at birth. This is due to a more efficient N retention on low protein intake and a concomitant transfer of protein from the maternal body of the foetal tissues[5].

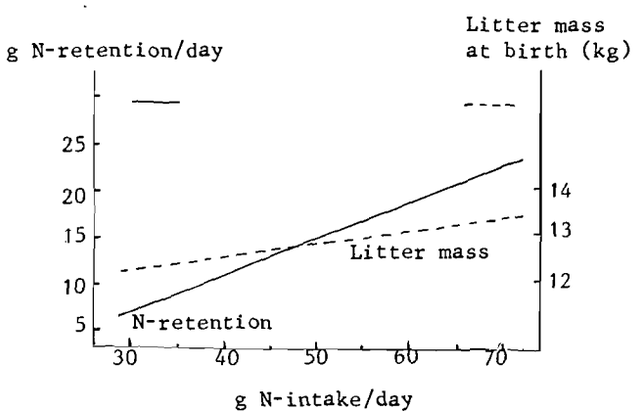

Fig. 3. Relation between N-intake during pregnancy and N-retention of litter mass at birth[6].

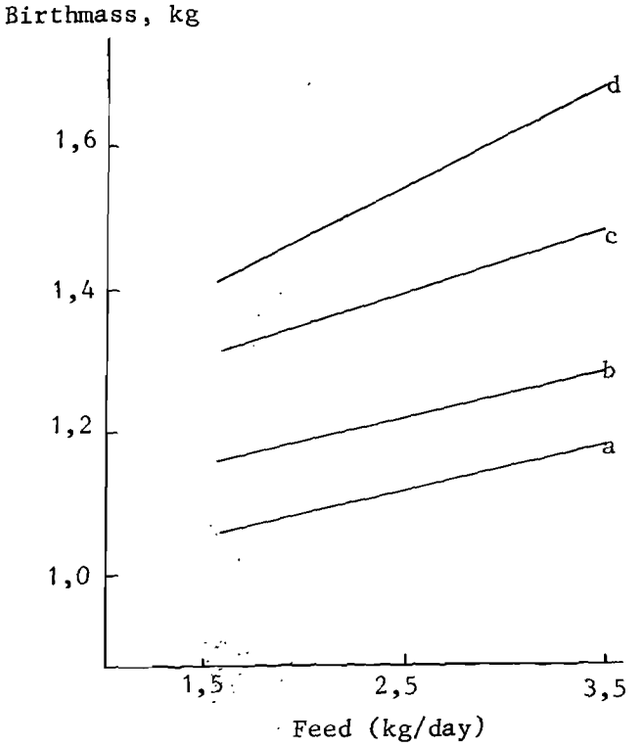

Fig. 4. Relation between the feeding level during pregnancy and the birthmass of piglets in 4 different herds (a, b, c, d)[17].

Fig. 4. demonstrates the effect of higher feed intakes on the mass of the piglets at birth. It is a common observation that the mass of the piglets increased with feeding level of the sow. A high litter mass at birth is not always desirable, because a mass considerably beyond the critical one of 1,2 to 1,3 kg often results in a lesser number of piglets (Fig. 2.) with an increased risk of dystocia. There is an optimal physiological as well as economical level between a greater number of less viable piglets and a lesser number of highly viable ones.

This optimum differs from herd to herd. Adaptation of feeding schedules can help to determine this optimum.

CONCLUSION

No universally applicable feeding standards for gilts and sows exist. There are too many variables which modify the nutritional requirements. It is important to know the needs of an average sow, as deduced from experimental data. It is even more important to know which are the variables that influence these needs and to quantify the impact of such variables. It is not surprising that recommendations, sound for one herd, may bode ill for another. The ultimate aim is long-term economically optimal efficiency.

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PLAGUE – WASHINGTON

A 19-year-old man went deer hunting with friends and relative on November 15, 1977. Two days later, while on the trail, he found a particularly dismembered dead rabbit. He amputated the front paws for good luck charms and gave them to another hunter in the party.

The young man, an automobile mechanic, handled the rabbit with his bare hands, which were scratched and bruised. On November 19 he noted festering sores on his hands, legs, and knees. Spiking fevers followed 24 hours later. He was cared for at home until December 11, when his physician admitted him to a local hospital because of continued bouts of fever and a weight loss of 10 pounds.

Initial white blood counts showed 8,400 cells/mm^3 with a normal differential pattern. Chest X-rays showed a right superior mediastinal mass with hilar adenopathy and no evidence of peripheral pneumonitis. Because Hodgkin's disease was suspected, a mediastinoscopy with mediastinal needle biopsy was performed. Results indicated the presence of necrotizing granuloma. By December 14 the fever had subsided, and the patient was discharged.

A blood specimen obtained on December 16 was sent to the state's public health laboratory for agglutination tests for *Francisella tularensis, Brucella,* and *Proteus.* Because of a high titer for *F. tularensis* (1:20,480), an epidemiologic investigation was begun.

A 10-day course of tetracycline was started on December 2. Despite repeated attempts to elicit a history of exposure to wild rabbits, none was obtained until after Christmas, when the grandfather remembered the rabbit paw incident. On January 3, a second blood specimen showed no decline in agglutination titer. The patient had refused to have further blood studies. He remains well with no evidence of relapse.

The person who received the "good luck charms" remains well. He has discarded the paws, however, so they could not be recovered and tested.

It is presumed that the man's illness resulted from exposure to the dead rabbit and that the portal of entry was primarily through traumatic skin breaks on the hands, with secondary mediastinal involvement. However, simultaneous inhalation of aerosolized *F. tularensis* cannot be ruled out.

Source: Center for Disease Control: Pneumonic tularemia, Washington. Morbidity Mortality Weekly Rep 27:105–106, 1978.

CASE REPORT **GEVALVERSLAG**

NUTRITIONAL MYOPATHY IN A DOG

I.B.J. VAN RENSBURG* AND W.J.A. VENNING†

ABSTRACT: Van Rensburg I.B.J.; Venning W.J.A. **Nutritional myopathy in a dog.** *Journal of the South African Veterinary Association.* (1979) **50,** No. 2 119 Dept. of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Rep. of South Africa.

A case of cardiac myopathy resembling Selenium-Vitamin E Deficiency myopathy is described in a puppy. The left ventricular myocardium was extensively involved. The macroscopic and microscopic appearances are described. The literature on the subject is briefly reviewed.

LITERATURE REVIEW

Canine myopathy is a rare finding[3 5]. The situation in South Africa is no different from this. However, experimental and natural cases of skeletal and cardiac myopathies due to a selenium (Se) and/or vitamin E (Vit. E) deficiency have been reported. The first publication was by Meier in 1958 who described a case in a 10 day old puppy where especially the tongue, neck and shoulder muscles were affected but the intercostal muscles, diaphragm and myocardium were also involved[5]. In 1963 Mankletow published a report on fatal congenital myocardial necrosis in two litters of pups and polymopathy in an adult dog fed on a diet consisting mainly of mutton from sheep amonst which white muscle disease was a problem[4]. He concluded that this was due to a dietary Se deficiency. In the same year nutritional myodegeneration was reported in a litter of Beagles by Kaspar & Lombard[3]. In this instance the left ventricular myocardium and skeletal musculature were involved. The histopathological lesions were typically those associated with white muscle disease, i.e. marked Zenker's hyaline degeneration and necrosis of striated muscles with outspoken calcification of the affected fibres. Hayes, Nielson & Rousseau made some interesting observations on experimental Vit. E deficient dogs[1]. They found an increased tendency towards haemolysis; a lipofuchs staining of the smooth muscles – especially in the intestinal, urinary bladder and arterial walls; neuroaxonal dystrophy, myodegeneration and replacement of sarcoplasm by fat. They further established a directly positive correlation between the tocopherol requirement and the consumption of polyunsaturated fatty acids. In 1970 severe smooth muscle lipofuchsinosis was again repoted by Hayes, Rousseau & Hegsted who actually monitored the plasma tocopherol levels and proved it deficient in affected individuals[2]. In this trial retinal degeneration was added to the list of lesions associated with a Vit. E deficiency. They concluded that Vit. E deficiency is more associated with lipofuchsinosis while Se deficiency was held responsible for muscular dystrophy. This view was supported by the findings of Van Vleet in an experiment with Vit. E and Se deficient diets in dogs[9]. This author further described renal mineralisation in dogs with skeletal myopathy as well as a prominent vascular fibrinoid necrosis in his cases. Contradictory to this however Tvedten & Trapp found severe myopathy in a dead pup as well as in muscle biopsies from a clinically affected litter mate which responded dramatically to Vit. E therapy[8]. Money, Pullan & Staples associated severe lung oedema and haemorrhage in pups with a Vit. E deficiency[6].

CASE REPORT

A seven-week old male Boxer pup was presented for post mortem examination with a history of difficult respiration, especially after mealtimes, gagging and sometimes vomition. According to the owner the pup had been kept on a diet of Epol crumbs** and mince meat. The breeder reported that its mother was kept on a ration of Beefy meat*** and Epol meal**, which during the last month of pregnancy were supplemented with Dogs delight‡ and sunflower oil. The pup in question was the smallest from the litter – but at no stage were any signs of muscular weakness noticed.

Grossly there was a severe lung oedema, the trachea being filled with white froth. The liver had a nutmeg appearance, was very congested and a few fibrin strands were loosely attached between some of the lobes. The left ventricular myocardium showed extensive, degeneration and calcification of the subendocardeal inner third of the myocardium, which had a white chalky appearance. The skeletal muscles showed general palor but no evidence of calcification or streakiness was noticed. The smooth muscles had a normal colour not revealing any evidence of lipofuchsinosis.

Histopathological examination revealed the most significant lesions to be present in the myocardium. These were limited to the macroscopically affected areas and consisted of conspicious areas of necrosis and mineralisation of muscle fibres. These areas stained a blueish-black with van Kossa stain. Some fibres were fragmented. The degree of calcification, the presence of which was confirmed by specific staining procedures, varied from an intense purplish homogenous mass to scattered purple granules in some fibres while in a few

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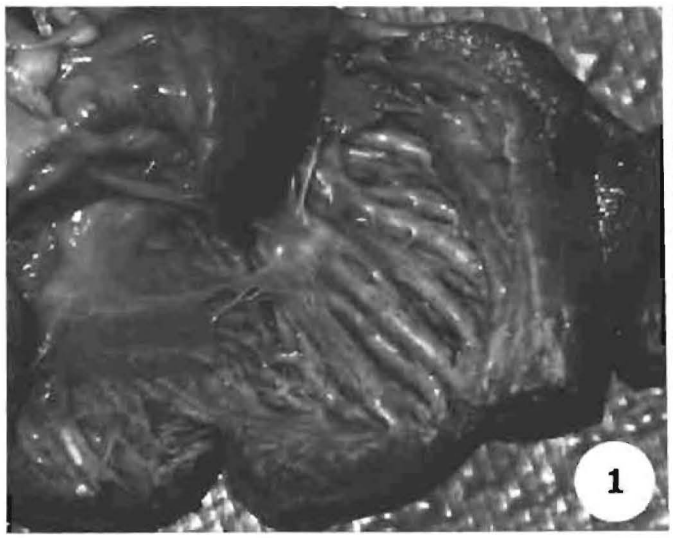

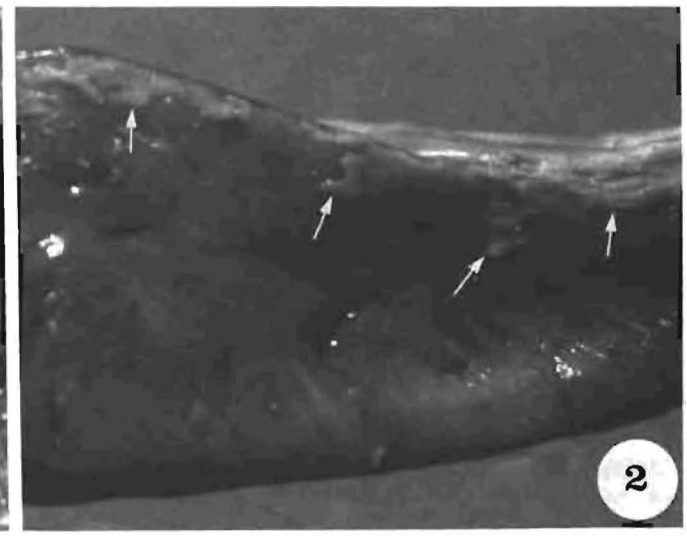

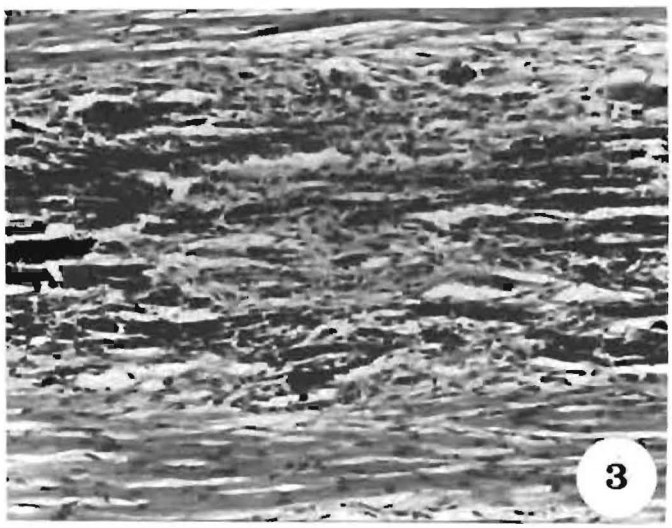

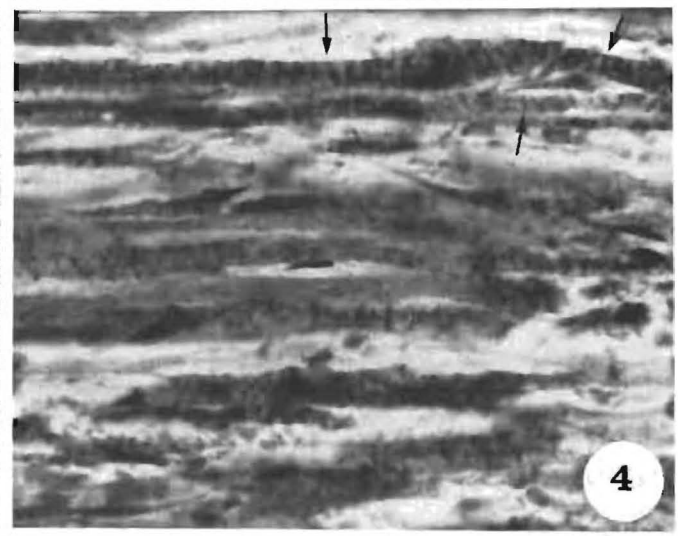

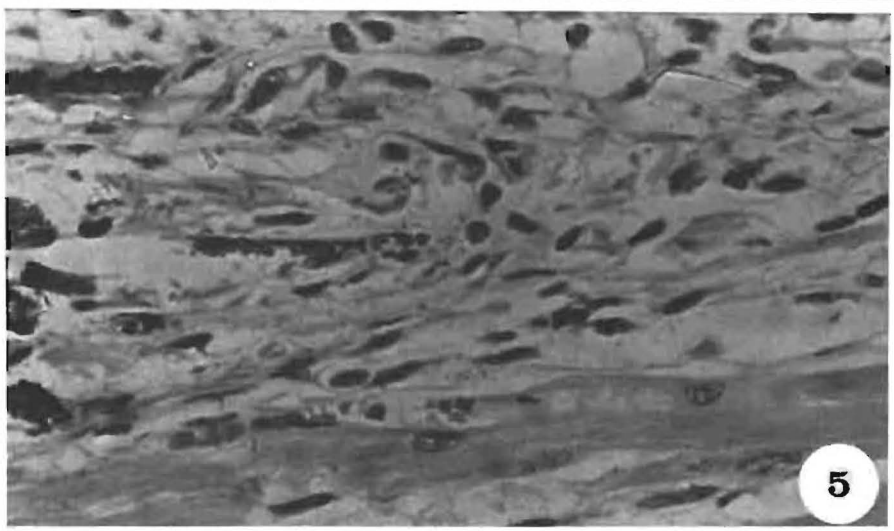

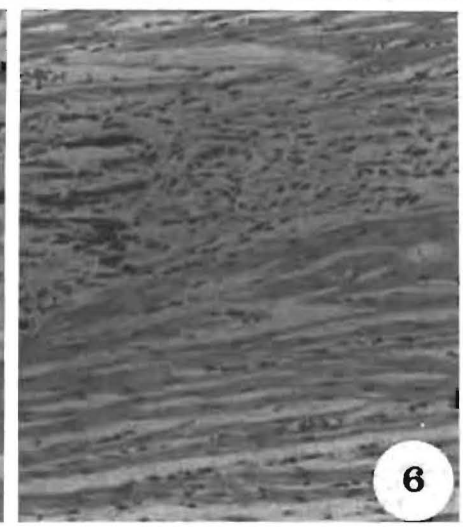

Fig. 1. Subendocardeal calcification of left ventricular myocardium

Fig. 2. Same as above – cut surface of papillary muscle.

Fig. 3. Necrosis, fragmentation and calcification of myocardium. HE x 200.

Fig. 4. Calcification of muscle fibres, prominent in Z-band area) arrows). HE x 800.

Fig. 5. As in Fig. 3 x 800.

Fig. 6. Early fibrous replacement of affected myocardium. HE x 200.

fibres thick purplish cross striations of calcium salts were deposited apparently in the area of the Z-bands stretching some distance into the I-bands in both directions (Fig. 4). In some fragments of fibres there was a proliferation of sarcolemmal nuclei lending a giant cell appearance to it. Some fibres were narrowed and col-

lapsed with disappearance of the sarcoplasm. There was a mild cellular infiltration of a few round cells, macrophages and fibroblasts into the affected areas. Evidence of repair was seen as small focal areas of early fibrosis (confirmed by staining with von Giesson). Outspoken hyalinisation of myocardial fibres was not a prominent feature in this case.

Sections from skeletal muscles and diaphragm did not show obvious Zenker's hyaline degeneration and necrosis or calcification although in isolated instances the cross striations interpreted as the Z-band area were widened and stained basophilic apparently as a result of early calcification. The muscles of the tongue showed a variation in diameter of the muscle fibres on cross section and a mild increase of interstitial histiocytes. The muscular layer of the oesophagus had a normal appearance. Periacinar necrosis, degeneration and congestion together with mild early centrilobular fibrosis was seen in the liver, while the lungs were very oedematous with mild activation of alveolar macrophages.

No significant lesions were noticed in sections of the spleen, lymph nodes, kidneys, pancreas and brain.

DISCUSSION

No proof exists that this was a case of congenital cardiac myopathy due to a Se-Vit, E deficiency. However, the resemblance of the lesions to those of lambs suffering from white muscle disease is so marked, that one must at least consider that similar nutritional deficiencies played a role.

The fact that they myocardium especially was involved while lipofuchsin accumulation in the smooth muscles was not noticed, suggests that this was probably more a case of Se deficiency rather than Vit. E; as suggested by the work of Hayes et al[2]. Nutritional myopathy is said to occur more readily in the most active muscles[7]. As the myocardiumis probably the most active muscle in the unborn animal, it can be concluded that this was a case of congenital cardiac myopathy. Enquiries however did not reveal similar problems in the rest of the litter.

In this case only the left ventricular myocardium was envolved. This is in accordance with the findings of Kaspar & Lombard[3] while Manklettow[4] reported lesions in the ventricular myocardium without specifying which ventricles were involved. The severe lung oedema and the early cyanotic induration of the liver suggests that the animal had suffered from a congestive heart failure.

The lesions in the skeletal muscles, diaphragm and tongue were very unimpressive. The only definite lesion seems to be the isolated fibres showing basophilic staining and widening of what is suspected to be the Z-band area. Van Vleet and co-workers[10] in an untrastructural study of cardiomyopathy of Se Vit. E deficient swine found "smearing" of the Z-bands to be an early lesion. In contrast to our finding other workers reported more conspicious lesions in the most active skeletal muscles (especially of the tongue, shoulder and neck muscles) than in the myocardium[1 5].

Although very little is known about the exact Se Vit. E requirements of the pregnant bitch, this case proves that the matter needs attention.

Dogs are definiately susceptible to myopathy due to deficiency of these nutrients, and this should be borne in mind by manufacturers of animal rations as well as by clinicians faced with pups suffering from disorders of the myocardium and skeletal musculature.

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FEATURE PAGE **TREFFERBLAD**

INTERTWINING OF HORNS OF FIGHTING BLACK WILDEBEESTE BULLS

This phenomenon, as depicted on page 102 of volume 44 of this Journal, is by no means rare in kuddu bulls. As far as could be ascertained, this was unknown up till now in the black wildebeest.

The chances of horn intertwining during fighting should be relatively much less in the case of the latter species, when one considers the difference in horn shape. Yet it can happen. As a matter of fact, the reciprocal movements to cause interlocking in the black wildebeest are relatively simple: dropping of the head and then jerking upwards by the one opponent, during the second phase of this movement the other opponent lunges forward and drops its head. Disengagement should also be relatively easy, which could explain the rarity of this phenomenon in this species. Only when the reciprocal actions of the fightting partners are well timed and executed with adequate force the horn of the one can be jammed between the horn and head of the other, as shown clearly in the accompanying photographs; and to such a degree that the opposing bulls are unable to force themselves free. In this species one should then speak of jamming rather than intertwining. Once jammed, the opponents would tend to pull away from one another and thus exert force in a horisontal plane, which would be futile.

This phenomenon was encountered and photographed in the winter of 1977 in the Willem Pretorius Reserve by the submitter. It probably was the result of a territorial fight.

Submission: Dr. M. Bootsma, Department of Nature Conservation, Provincial Administration of the O.F.S., P.O. Box 517, Bloemfontein, 9300.

INEENSTRENGELING VAN HORINGS BY VEGTENDE SWARTWILDEBEESBULLE

Hierdie verskynsel soos uitgebeeld op bladsy 102 van volume 44 van hierdie tydskrif, is geensins seldsaam by koedoebulle nie. Sover vasgestel kon word was dit tot dusver onbekend by die swartwildebees. In vergelyking met die kans vir ineenstrengeling gedurende 'n geveg tussen koedoebulle, sou mens reken dat die moontlikheid by die swartwildebees baie kleiner sou wees weens laasgenoemde se horingfatsoen. Tog kan dit gebeur.

Trouens, die resiprokale bewegings om 'n sluiting te veroorsaak by die swartwildebees is betreklik eenvoudig: 'n laat sak van die kop en dan opwaartse beweging van die een opponent, terwyl die ander een gedurende die tweede fase van so 'n beweging vorentoe beur en sy kop laat sak. Ontsluiting behoort ook betreklik maklik te wees, wat eintlik die seldsaamheid van die verskynsel by hierdie dieresoort kan verklaar. Slegs as die resiprokale bewegings van die vegtende pare goed gesinkroniseer is en met genoeg krag uitgevoer word, kan die horing van die een tussen die kop en horing van die ander vasgeknel word, soos die bygaande fotos duidelik toon en wel tot so 'n mate dat hulle nie by magte is om los te ruk nie. By hierdie diersoort sou mens dus eerder van vasknelling as van ineenstrengeling kan praat. Is die diere eenmaal vasgeknel, dan sou hulle geneig wees om van mekaar weg te ruk en dus horisontale krag uit te oefen, wat futiel sou wees.

Hierdie verskynsel is in die winter van 1977 in die Willem Pretorius-wildtuin in die Oranje Vrystaat deur die insender teengekom en gefotografeer. Dit was waarskynlik die gevolg van 'n territoriale geveg.

Insender: Dr. M. Bootsma, Afdeling Natuurbewaring, Provinsiale Administrasie van die O.V.S., Posbus 517, Bloemfontein, 9300.

MONITORING OF BACTERIOLOGICAL CONTAMINATION AND ASSESSMENT OF CARCASE SURFACE GROWTH BY USING DIRECT AND INDIRECT CONTACT EXAMINATION TECHNIQUES AND VARIOUS COLONY COUNTING PROCEDURES

B. McCULLOCH and C.J. WHITEHEAD

ABSTRACT: McCulloch B.; Whitehead C.J. **Monitoring of bacteriological contamination and assessment of carcas surface growth by using direct and indirect contact examination techniques and various colony counting procedures.** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 123–133 (En.) Veterinary Laboratory, Division of Veterinary Services, P.O. Box 41, Grahamstown 6140, Republic of South Africa.

Two hundred and sixty nine beef, 230 sheep and 165 pig carcase surface were examined bacteriologically. Direct and indirect contact examination techniques were utilised. Colony counts per cm² were expressed in geometric progression. Counting procedures, direct and indirect contact examinations, and effects of chilling were considered. Subsequently, results from an additional 489 beef, 520 sheep, and 408 pig carcases were employed to illustrate a count classification arrangement against which bacteriological monitoring assessments could be measured.

INTRODUCTION

Bacteria in meat and meat products constitute a health hazard and affect keeping qualities. Bacteria may be found in the tissues of sick animals. By and large, however, most bacterial become attached to carcases during slaughter and storage. Both pathogenic and spoilage bacteria require consideration[2 4 5]. Fungi also cause spoilage but as access to the carcase usually occurs during storage, further consideration of fungi has been ignored for the sake of simplicity. A variety of selective procedures are available for determination of pathogenic bacterial forms; many of the procedures are based on "present" or "absent" criteria[7 8 9 10 11 12]. The overall weight of the growth of pathogenic and spoilage bacterial is usually expressed numerically and arrived at by some form of counting procedure. Counting procedures can be affected in various ways namely; excision and homogenization, scraping, swabbing, washing, direct contact and indirect contact examinations. Each examination method has advantages and disadvantages; in broad terms the greater the "accuracy" the greater the required expenditure in time labour, materials and equipment.

In this paper, counting procedures, direct and indirect examination techniques and the effects of chilling are considered initially. Subsequent indirect contact examination of warm carcases, modified counting procedure and geometric arrangement of colony counts per cm² are utilised in a monitoring assessment.

MATERIALS AND METHODS

Beef, sheep and pig carcase surfaces were examined bacteriologically at Grahamstown and Port Elizabeth abattoirs. Warm surfaces were examined at Grahamstown; warm and chilled at Port Elizabeth. Direct and indirect contact examination techniques were utilised at both places.

(a) Direct and indirect contact examination techniques and culture method

Direct contact examinations were affected on plate count agar (Oxoid) in commercially available contact plates ("Rhodac"). Indirect contact examinations were made by means of a sterile universal container bottle top (surface area 5,50 cm²), which while attached to the bottle was applied to the carcase surface and then carried over, in groups of four, to plate count agar in standard petri-dishes (Fig. 1).

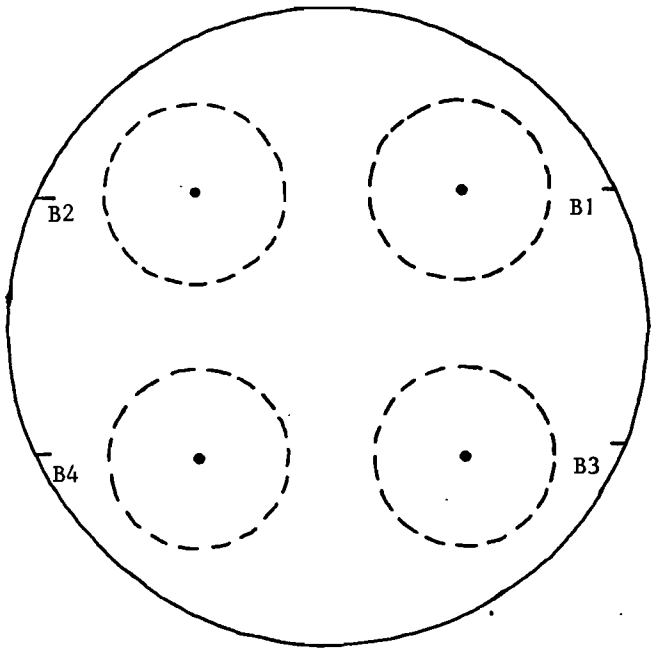

Fig. 1. The spacing of bottle top impressions (broken lines) on plate count agar. Plate marking system also shown; B1 = beef carcase 1, central dot provided to guide placing of impression.

Examinations of warm carcases were carried out as they came off the line; on the following day the same carcases were examined after about 20 hours chilling to a temperature of about 9°C. External surfaces were examined; the site of all examinations was just behind the left shoulder close to the axilla and elbow joint.

Plates were incubated aeriobically for 20 hours at 37°C.

(b) Counting procedures

Colonies were counted with the aid of a stereomicroscope and grid outline as in Fig. 2.; the grid was attached to the glass insert of the microscope stage. The number of colonies within the circle, corresponding to the size of the universal bottle top, was determined.

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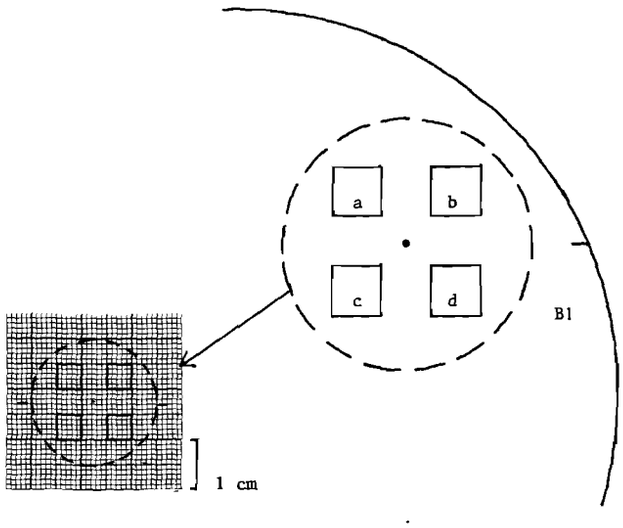

Fig. 2. Grid and positioning of plate for colony number determination.

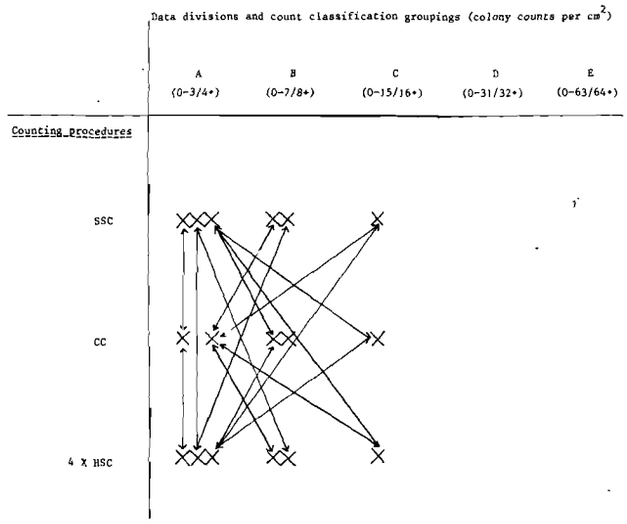

Fig. 3. Range of chi-squared testing at data divisions and count classification groupings.

The number of colonies in each of the 0,25 cm² squares a, b, c and d were also determined and recorded.

Three different direct and indirect colony counts per cm² were obtained as follows:

The Circle Count (CC), by division of the number of colonies in the circle by the area of the circle (5,50 cm²);

The Square Sum Count (SSC), by addition of the number of colonies in the four 0,25 cm² squares a, b, c and d; and

The 4 x Highest Square Count (4 x HSC), by multiplication of the highest number of colonies in square 1, b, c or d by factor 4.

(c) Count classifications, data divisions and statistical analyses

Colony counts per cm², as determined by the CC, SSC and 4 x HSC procedures were separately classified in geometric progression, first term/common ration – 4/2; count classifications were: I (colony counts per cm²; 0–3), II (colony counts per cm²; 4–7), III (colony counts per cm²; 8–15), IV (colony counts per cm²; 16–31), etc. Colony numbers too dense and too numerous to count were allocated at the time of assessment to the highest count classification of the species concerned.

Count classification data were grouped together for further analysis. Data divisions lay between count classifications; all the data on each side of the relevant division were then grouped together. Thus, data division A lay at count classifications I/II and the count classification groupings were colony counts per cm² 0–3/4+, data division B lay at count classifications II/III and count classification groupings were colony counts per cm² 0–7/8+. Data divisions C and D etc., followed the same pattern; totals as expressed at each data division were accordingly constant.

Differences between the counting procedures were assessed by chi-squared tests as indicated by lines running between data divisions A (colony counts per cm²; 0–3/4+), B (colony counts per cm²; 0–7/8+) and C (colony counts per cm²; 0–15/16+) in Fig. 3. Analysis were repeated between data divisions B (colony counts per cm²; 0–7/8+), C (colony counts per cm²; 0–15/16+) and D (colony counts per cm²; 0–31/32+) and likewise between C, D and E etc. Actual evaluation of the counting procedures was indicated by the lines running between the data divisions of Fig.4.

Differences between direct and indirect contact examination techniques and between warm and chilled carcase studies were evaluated by chi-squared tests as indicated by the lines running between the data divisions of fig. 5 and 6 respectively.

Regression analysis and unclassified CC, SSC and/or 4 x HSC colony counts per cm² were employed in supplementary assessment of the counting procedures, the direct and indirect contact examinations and the warm and chilled carcase studies (Tables 1, 2 and 3). For this purpose colony numbers too dense to assess accurately were estimated at the mid count value of the count classification to which they were allocated. In repeat regression analysis, paired zero values and estimated counts per cm² were discarded from assessment (Tables 1, 2 and 3).

(d) The CC, SSC and 4 x HSC counting procedures, and count classifications (Port Elizabeth: beef carcases 180, sheep 180, pig 120).

Indirect contact examinations of warm carcase surfaces were utilised to assess the effect of the CC, SSC and 4 x HSC counting procedures on the estimation of colony numbers as determined by count classification. Classifications were evaluated by chi-squared tests and unclassified data by regression analysis (Fig. 4 Table 1 respectively).

(e) Direct contact examinations, indirect contact examinations and count classifications (Grahamstown: beef carcases 89, sheep 50, pig 45).

Warm carcase examinations and the 4 x HSC counting procedure were utilised to consider the effect of direct contact and indirect contact examination techniques on the estimation of colony numbers as determined by count classification. Classifications were evaluated by chi-squared tests and unclassified data by regression analysis (Fig. 5 and Table 2 respectively).

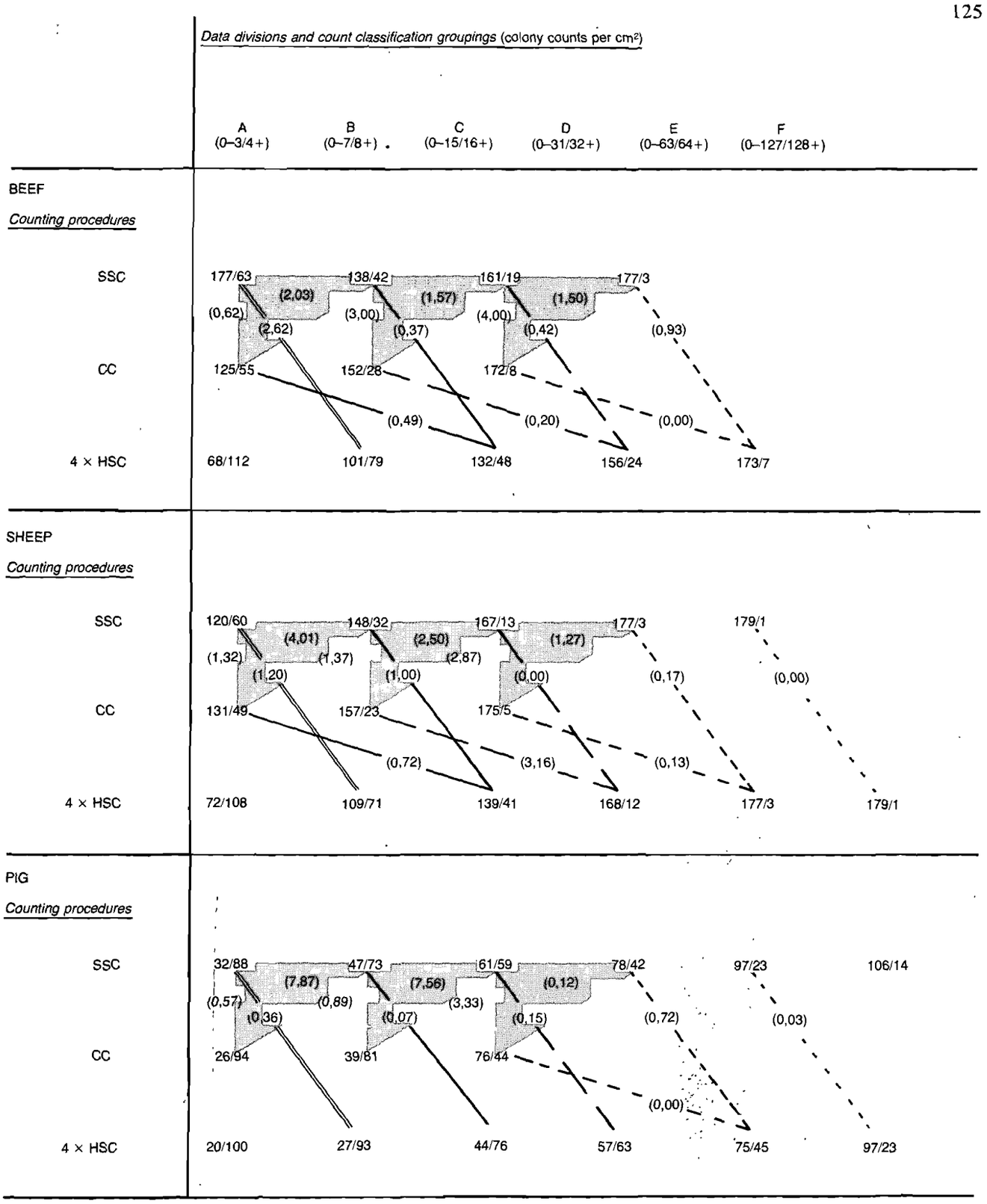

Fig. 4 Beef, sheep and pig carcases; approximation of counting procedures as indicated by chi-squared tests at data divisions (indirect contact, warm carcase, examinations – chi-squared values in brackets).

(f) Chilling and count classifications (Port Elizabeth: beef carcases 180, sheep 180, pig 120)

Indirect contact examination technique and the 4 x HSC counting procedure were utilised to assess the effect of chilling on the estimation of colony numbers as determined by count classification. Classifications were evaluated by chi-squared tests and unclassified data by regression analysis (Fig. 6 and Table 3 respectively).

Table 1: REGRESSION ANALYSIS STUDIES: INDIRECT CONTACT, WARM CARCASE, UNCLASSIFIED COLONY COUNTS PER CM² – CC, SSC AND 4 X HSC COUNTING PROCEDURES

Counting procedures	Species	Carcases counted			Regression analysis*; zero and estimated counts included			Regression analysis*; zero and estimated counts excluded		
		Total	paired zero counts	estimated counts	n	r	$\frac{r}{S.E.\ of\ r}$	n	r	$\frac{r}{S.E.\ of\ r}$
CC/SSC	Beef	180	25	4	180	0,8774 P<0,001	11,74 significant	151	0,8704 P<0,001	10,66 significant
	Sheep	180	17	3	180	0,9012 P<0,001	12,06 significant	160	0,9051 P<0,001	11,41 significant
	Pig	120	1	55	120	0,7014 P<0,001	7,65 significant	64	0,8015 P<0,001	6,36 significant
CC/4 x HSC	Beef	180	25	4	180	0,8732 P<0,001	11,68 significant	151	0,8484 P<0,001	10,39 significant
	Sheep	180	17	3	180	0,8668 P<0,001	11,60 significant	160	0,8515 P<0,001	10,74 significant
	Pig	120	1	55	120	0,6789 P<0,001	7,41 significant	64	0,7366 P<0,001	5,85 significant
SSC/4 x HSC	Beef	180	68	0	180	0,9420 P<0,001	12,60 significant	112	0,9272 P<0,001	9,77 significant
	Sheep	180	72	1	180	0,9706 P<0,001	12,99 significant	107	0,9577 P<0,001	9,86 significant
	Pig	120	19	25	120	0,8496 P<0,001	9,27 significant	76	0,9398 P<0,001	8,14 significant

*P values of r were all noted as significant. Values of $\frac{r}{S.E.\ of\ r}$ greater than 2, were regarded as further evidence of significance; S.E. of r = $\frac{1}{\sqrt{n-1}}$

Table 2: REGRESSION ANALYSIS STUDIES: WARM CARCASE, UNCLASSIFIED COLONY COUNTS PER CM², 4 X HSC COUNTING PROCEDURE – DIRECT CONTACT AND INDIRECT CONTACT EXAMINATION TECHNIQUES

Examination techniques	Species	Carcases counted			Regression analysis*; zero and estimated counts included			Regression analysis*; zero and estimated counts excluded		
		Total	paired zero counts	estimated counts	n	r	$\frac{r}{S.E.\ of\ r}$	n	r	$\frac{r}{S.E.\ of\ r}$
Direct/	Beef	89	40	0	89	0,5777 P<0,001	5,42 significant	49	0,4895 P<0,001	3,39 significant
	Sheep	50	6	1	50	0,5536 P<0,001	3,88 significant	43	0,5382 P<0,001	3,49 significant
indirect	Pig	45	1	8	45	0,3862 P<0,01 P>0,001	2,56 significant	36	0,4445 P<0,01 P>0,001	2,63 significant

*P values of r were all noted as significant. Values of $\frac{r}{S.E.\ of\ r}$ greater than 2, were regarded as further evidence of significance; S.E. of r = $\frac{1}{\sqrt{n-1}}$

Table 3: REGRESSION ANALYSIS STUDIES: INDIRECT CONTACT, UNCLASSIFIED COLONY COUNTS PER CM², 4 X HSC COUNTING PROCEDURE – WARM CARCASE AND CHILLED CARCASE EXAMINATIONS

State of carcaces	Species	Carcases counted			Regression analysis*; zero and estimated counts included			Regression analysis*; zero and estimated counts excluded		
		Total	paired zero counts	estimated counts	n	r	$\frac{r}{S.E.\ of\ r}$	n	r	$\frac{r}{S.E.\ of\ r}$
Warm/	Beef	180	17	4	180	–0,04 insignificant	–0,53 insignificant	159	–0,05 insignificant	–0,65 insignificant
chilled	Sheep	180	45	1	180	–0,07 insignificant	–0,93 insignificant	134	–0,15 insignificant	–1,74 insignificant
	Pig	120	5	29	180	0,09 insignificant	1,00 insignificant	86	–0,01 insignificant	–0,05 insignificant

*P values of r were all noted as significant. Values of $\frac{r}{S.E.\ of\ r}$ greater than 2, were regarded as further evidence of significance; S.E. of r = $\frac{1}{\sqrt{n-1}}$

(g) Monitoring of bacteriological contamination; indirect contact, warm carcase, 4 x HSC count classifications (Port Elizabeth: beef carcases 489, sheep 520, pig 480)

After the preliminary observations were completed, bacteriological contamination of carcase surfaces was monitored over a six month trial period at Port Elizabeth abattoir.

Indirect contact examination of warm carcase surfaces and the 4 x HSC counting procedure were utilised to estimate colony counts per cm². Count classifications were determined. The number of carcases which fell into each count classification were expressed as a percentage of total carcase classifications undertaken on the date of examination (Table 4). The count classification percentage figures were also expressed by bargraph arrangement (Fig. 7).

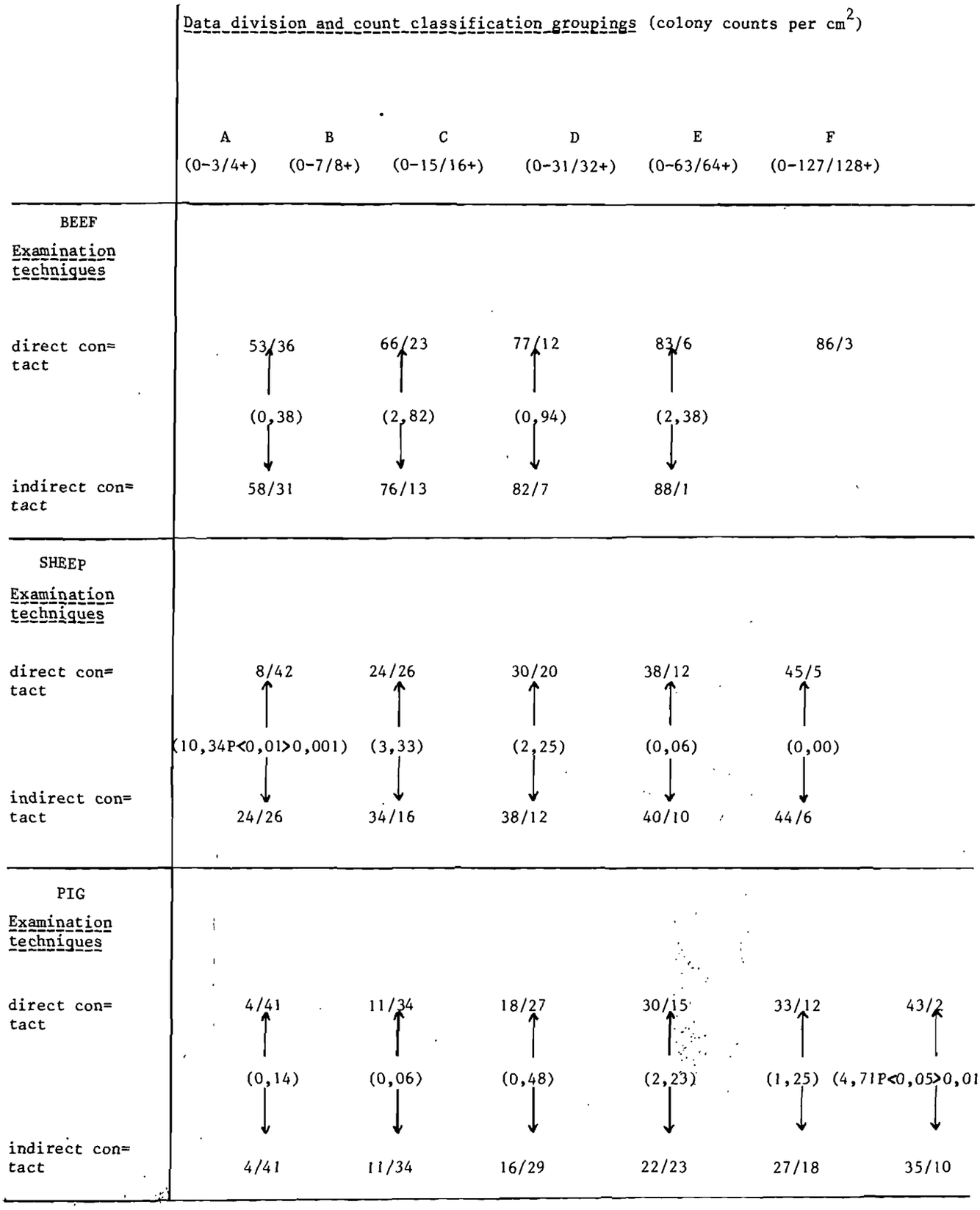

Fig. 5. Beef, sheep and pig carcases; approximation of direct contact and indirect contact examination techniques as indicated by chi-squared tests at data divisions (warm carcase examinations, 4 x HSC counting procedure chi-squared and P values in brackets).

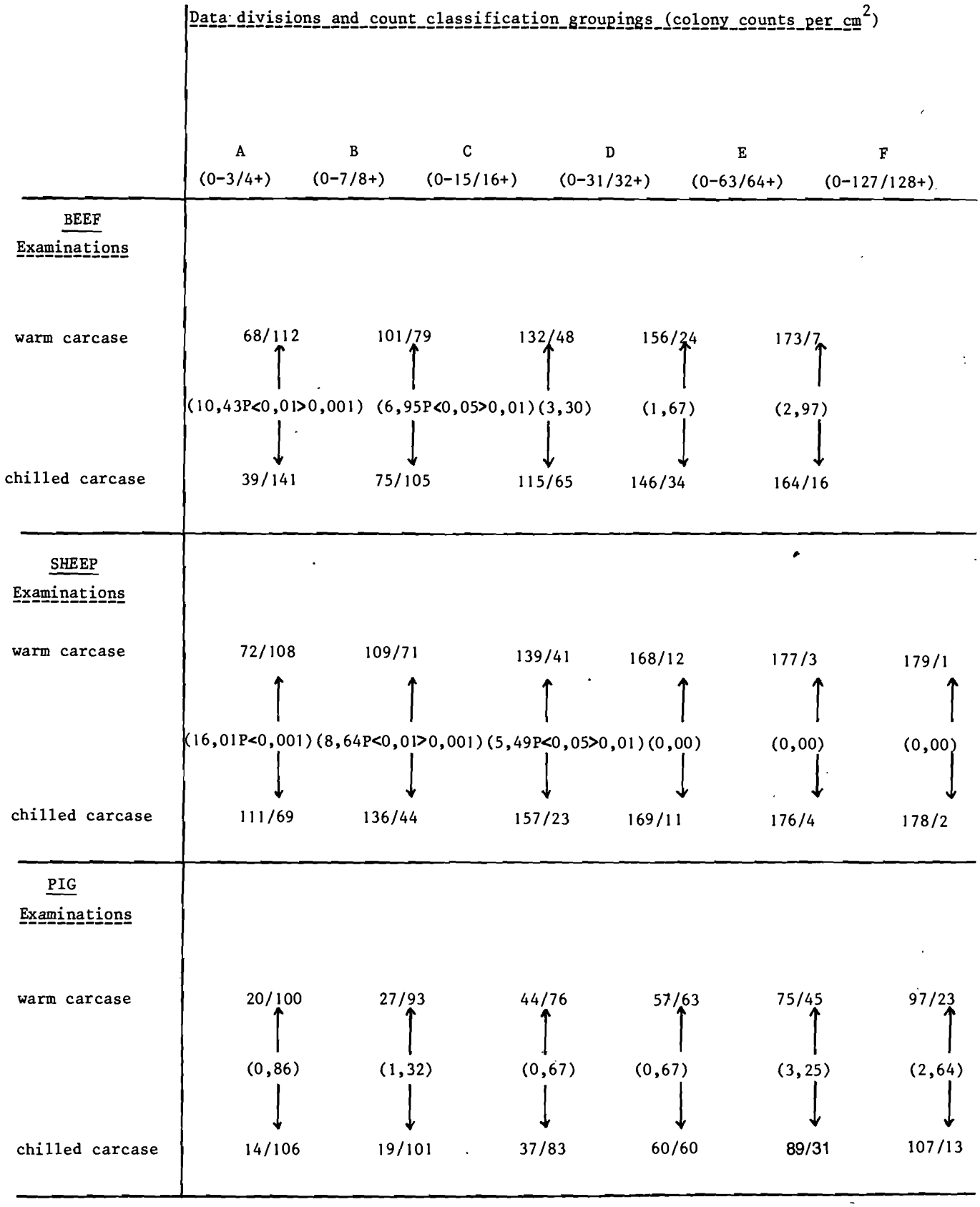

Fig. 6. Beef, sheep and pig carcases; assessment of warm carcase and chilled carcase examinations as indicated by chi-squared tests at data divisions (indirect contact examination technique, 4 x HSC counting procedure – chi-squared and P values in brackets).

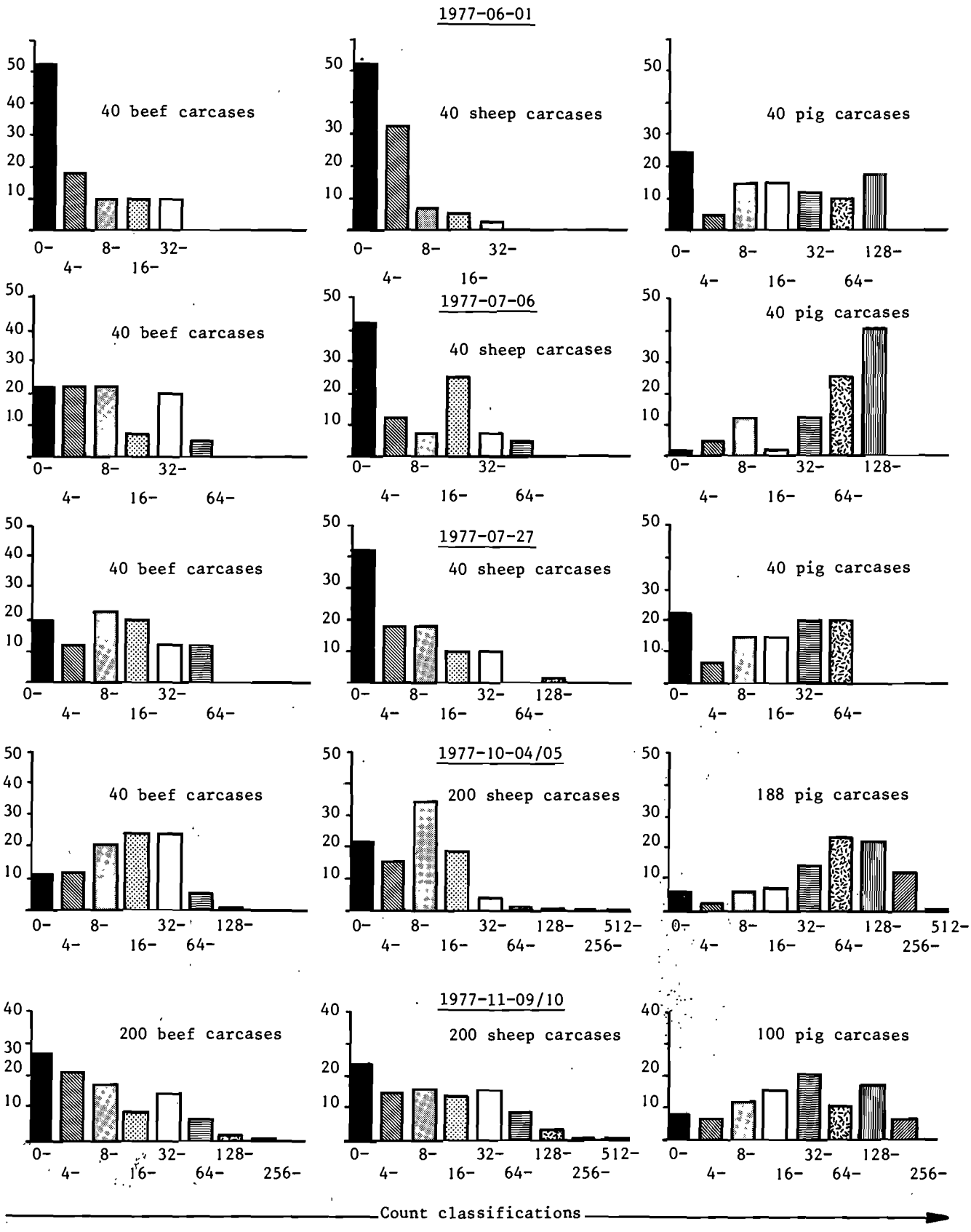

Fig. 7. Beef, sheep and pig carcases; bacteriological monitoring, bar-graph arrangements to illustrate density of bacterial growth (indirect contact, warm carcase, 4½x HSC, count classifications).

Table 4: MONITORING OF BACTERIOLOGICAL CONTAMINATION: COUNT CLASSIFICATIONS – INDIRECT CONTACT, WARM CARCASE, COLONY COUNTS PER CM², 4 X HSC COUNTING PROCEDURE

	Number and percentage of carcases in each count classification								
Species	Beef								
Count classifications	I	II	III	IV	V	VI	VII	VIII	IX
Colony counts per cm²	0–3	4–7	8–15	16–31	32–63	64–127	128–255	256–511	512–1023
1977-06-01	21 52,5%	7 17,5%	4 10,0%	4 10,0%	4 10,0%				
1977-07-06	9 22,5%	9 22,5%	9 22,5%	3 7,5%	8 20,0%	2 5,0%			
1977-07-27	8 20,0%	5 12,5%	9 22,5%	8 20,0%	5 12,5%	5 12,5%			
1977-10-4/5	19 11,2%	21 12,4%	34 20,1%	42 24,9%	41 24,3%	10 5,9%	2 1,2%		
1977-11-9/10	55 27,5%	42 21,0%	35 17,5%	19 9,5%	29 14,5%	14 7,0%	5 2,5%	1 0,5%	
Species	Sheep								
Count classifications	I	II	III	IV	V	VI	VII	VIII	IX
Colony counts per cm²	0–3	4–7	8–15	16–31	32–63	64–127	128–255	256–511	512–1023
1977-06-01	21 52,5%	13 32,5%	3 7,5%	2 5,0%	1 2,5%				
1977-07-06	17 42,5%	5 12,5%	3 7,5%	10 25,0%	3 7,5%	2 5,0%			
1977-07-27	17 42,5%	7 17,5%	7 17,5%	4 10,0%	4 10,0%	0	1 2,5%		
1977-10-4/5	44 22,0%	32 16,0%	69 34,5%	38 19,0%	9 4,5%	4 2,0%	2 1,0%	1 0,5%	1 0,5%
1977-11-9/10	48 24,0%	31 15,5%	33 16,5%	29 14,5%	32 16%	18 9,0%	7 3,5%	1 0,5%	1 0,5%
Species	Pig								
Count classifications	I	II	III	IV	V	VI	VII	VIII	IX
Colony counts per cm²	0–3	4–7	8–15	16–31	32–63	64–127	128–255	256–511	512–1023
1977-06-01	10 25,0%	2 5,0%	6 15,0%	6 15,0%	5 12,5%	4 10,0%	7 17,5%		
1977-07-06	1 2,5%	2 5,0%	5 12,5%	1 2,5%	5 12,5%	10 2,5%	16 40,0%		
1977-07-27	9 22,5%	3 7,5%	6 15,0%	6 15,0%	8 20,0%	8 20,0%			
1977-10-4/5	13 6,9%	7 3,7%	13 6,9%	15 8,0%	28 14,9%	44 23,4%	42 22,3%	24 12,8%	2 1,1%
1977-11-9/10	8 8,0%	7 7,0%	12 12,0%	16 16,0%	21 21,0%	11 11,0%	18 18,0%	7 7,0%	

RESULTS

(a) Counting procedures, count classifications and data divisions (Port Elizabeth: beef carcases 180, sheep 180, pigs 120).

The results of indirect contact, warm carcase examinations were utilised to consider the CC, the SSC and the 4 x HSC counting procedures in relation to data divisions and count classifications.

Chi-squared values were determined at the data divisions (Fig. 4). Non significant patterns of chi-squared values were noted in the beef, sheep and pig sections. It was seen that beef and sheep CC count classification groupings at data division A (colony counts per cm^2; 0–3/4+) were equivalent to 4 x HSC count classification groupings at data division C (colony counts per cm^2 0–15/16+) (the middle and bottom rows, Fig. 4, beef and sheep sections). Equivalent groupings in the pig section (Fig. 4) were excepted in that the relationship was more from data division A (colony counts per cm^2; 0–3/4+) to data division B (colony counts per cm^2; 0–7/8+). Notwithstanding the relative relationships of CC count classification groupings at data division A (colony counts per cm^2; 0–3/4+) and 4 x HSC count classification groupings at data division C (colony counts per cm^2; 0–15/16+) were maintained from data division to data division across Fig. 4 beef and sheep sections, and also, albeit to a lesser extent, to the right of the pig section.

Irrespective of species 4 x HSC count classification groupings at data division C (colony counts per cm^2; 0–15/16+) were approximate to SSC count classification groupings at data division B (colony counts per cm^2; 0–7/8+) (the bottom and top rows; fig. 4; beef, sheep and pig sections). Similar relative relationships were maintained from data division to data division in both directions across the three sections. The final linkages of SSC count classification groupings at data division B (colony counts per cm^2; 0–7/8+) to CC count classification groupings at data division A (colony counts per cm^2; 0–3/4+), although discernable in beef, were not convincing. In all species there were very close relationships between count classification groupings at the SSC data divisions and the same CC data divisions (the top and middle rows; Fig. 4; beef, sheep and pig sections). Specific linkage was not defined and the affected areas were shaded on Fig. 4.

Regression analysis studies of indirect contact, warm carcase. Unclassified colony counts per cm^2 showed close relationships between the CC, the SSC and the 4 x HSC counting procedures. The values of r were high at $P < 0{,}001$ levels (Table 1). Repeat analysis, which excluded paired zero values and estimated counts were in accord (Table 1).

(b) Direct contact examinations, indirect contact examinations and count classifications (Grahamstown: beef carcases 89, sheep 50, pig 45)

The results of direct and indirect contact examination of warm carcases by the 4 x HSC counting procedure were utilised to assess the effects of the direct and indirect contact examination techniques on count classification.

Chi-squared values were determined at the data divisions, Fig. 5. At data division A (colony counts per cm^2; 0–3/4+) in sheep and at data division F (colony counts per cm^2; 0–127/128+) in pigs there were indications that direct count classifications were higher than indirect ones ($P < 0{,}01 > 0{,}001$ and ($P < 0{,}05 > 0{,}01$ respectively): at the other sheep and pig data divisions and at all the beef data divisions count classification indications were approximately the same.

Regression analysis studies of direct and indirect contact, warm carcase, unclassified colony counts per cm^2, as assessed by the 4 x HSC counting procedure showed relationships between the direct and indirect contact examination techniques. The values of r, although only moderately high were at significant levels; beef $P < 0{,}001$, sheep $P < 0{,}001$, pig $P < 0{,}01 > 0{,}001$ (Table 2). Repeat analysis, which excluded paired zero-values and estimated counts were in accord (Table 2).

(c) Chilling and count classifications (Port Elizabeth: beef carcases 180, sheep 180, pig 120)

The results of indirect contact, colony counts per cm^2 of warm and chilled carcases as estimated by the 4 x HSC counting procedure were utilised to assess the effect of chilling on count classification.

Chi-squared values were determined at the data divisions (Fig. 6). At the lower levels of data division there were indications that after chilling there was a rise in beef count classifications ($P < 0{,}05 > 0{,}001$) and a drop in sheep count classification ($P < 0{,}05 > 0{,}001$): pig count classification indications remained largely unchanged at all levels of data division.

Regression analysis studies of the results of indirect contact, unclassified colony counts per cm^2 of warm and chilled carcases, as assessed by the 4 x HSC counting procedure, however, showed no significant relationship between the warm and chilled results. The values of r were low; beef – 0,04, sheep – 0,07, pig 0,09 (Table 3). Repeat analysis which excluded paired zero values and estimated counts were in accord (Table 3).

(d) Monitoring of bacteriological contamination: indirect contact, warm carcase, 4 x HSC count classifications (Port Elizabeth: beef carcases 489, sheep 520, pig 480)

The results of indirect contact, warm carcase, 4 x HSC count classifications were used in bacteriological monitoring assessment. Patterns of contamination were monitored over a six month trial period. Count classifications were expressed in percentage form by table and bar-graph arrangements. Count classification percentage figures over the period were better for beef and sheep than pig; count classification I (colony counts per cm^2; 0–3) ranged from beef 11,2 % –52,5 %, sheep 22,0 % – 52,5 %, pig 2,5 % – 25,0 %, count classification VI (colony counts per cm^2; 64-127) ranged from beef 0 %– 12,5 %, sheep 0 % – 9,0 %, pig 2,5 % – 23,4 % (Table 4, Fig. 7). By and large, only results from pig carcases fell particularly into the very high count classification ranges, such as, count classification VII (colony counts per cm^2; 128–255) and count classification VIII (colony counts per cm^2; 255–511) (Table 4, Fig. 7).

DISCUSSION

(a) Counting procedures, count classifications and data divisions (Port Elizabeth: beef carcases 180, sheep 180, pig 120)

In the initial stages the CC, the SSC and the 4 x HSC per cm^2 counting procedures were evaluated. Numbers of colonies actually counted varied considerably according to the counting procedure. At count classification V (colony counts per cm^2; 32–63) levels of the CC counting procedure, some 180–350 individual colonies were enumerated at one time. Indeed colony densities about the 200 mark were regarded as almost too numerous to determine. Equivalent count classification V numbers, i.e. individual colonies enumerated for the SSC and the 4 x HSC counting procedures were 32–63 and 8–15, higher numbers were readily determined but, in fact, did not exceed the outer limits at count classification VII (colony counts per cm^2; 128–255) of 255 and 63 respectively.

The counts were classified by geometric progression, a form of logarithmic expression. The bulking up effect of geometric progression can be achieved by conversion to logarithmics, a not uncommon practice[1 2 3 6]. Geometric progressions however are probably more descriptive of the actual situation than logarithmic conversions. Geometric progression as distinct from arithmetic progression ensured adequate recognition of the importance of low and high colony count values and was effective in reducing the number of colony count classification and data divisions which required to be reviewed.

Examination of data divisions fig. 4, showed as indicated by the lines within the figure, a fairly consistent relationship between the three counting procedures, in that, the observed values of chi-squared did not depart significantly from those expected on the null hypothesis. Within geometric progression it appeared, therefore, that the monotony, tedium and fatigue associated with the CC counting procedure were scarcely justified. Regression analysis studies on unclassified colony counts per cm^2 were in agreement as were repeat ones which excluded paired zero values and estimated counts from analysis. In this respect estimated values, especially of pigs, were particularly affected by dense growth as illustrated in Table 1 and to a lesser extent in Tables 2 and 3.

(b) The effect direct and indirect contact examinations on count classifications (Grahamstown: beef carcases 89, sheep 50, pig 45)

Comparisons of direct and indirect contact examinations count classifications were determined from 4 x HSC counting procedures (Fig. 5). Apart from sheep and pig differences at extremes of data division A (colony counts per cm^2; 0–3/4+) and F (colony counts per cm^2; 0–127/128+) respectively, the indications were that the direct and indirect contact examinations count classifications were in approximate relationship. Regression analysis studies on unclassified colony counts per cm^2 were in broad agreement (Table 2).

(c) The effect of chilling on count classifications (Port Elizabeth: beef carcases 180, sheep 180, pig 120)

The effects of chilling on beef, sheep and pig count classifications were also determined from 4 x HSC counting procedures (Fig. 6). Indications were that at the lower data divisions, beef and sheep count classifications rose and dropped respectively and that pig ones remained fairly static throughout. No attempts were made to determine the reasons for these apparent differences. Beef, sheep and pig carcases were stored in separate chillers and many factors could have played a role. Results of regression analysis studies on unclassified colony counts per cm^2 showed no warm and chilled carcase relationship (Table 3).

(d) Monitoring of bacteriological contamination, indirect contact, warm carcase, 4 x HSC count classifications (Port Elizabeth: beef carcases 489, sheep 520, pig 480)

Indirect contact, warm carcase 4 x HSC count classifications were used in bacteriological monitoring assessment (Table 4, Fig. 7). As might be expected, greatest bacterial growth was seen in pigs and least in sheep and cattle, with the position possibly slightly better in sheep than in cattle. No endeavour was made to explain the widening of the count classification range which occurred in all species over the six month trial period. Increase in sample size from 40 to 200 carcases, concentration on indirect contact warm carcase, 4 x HSC count classifications and hygienic considerations could have played roles. However, irrespective of their significance, species variation and month to month changes were illustrated. It was felt that the bar-graph arrangements of Fig. 7 demonstrated these observations with effect, and in this respect were more expressive than the tabulated results in Table 4.

(e) Bacteriological contaminaion in the meat industry

A number of circumstances need consideration when the problems of bacteriological monitoring of meat are assessed. For example, various factors influence determination of individual bacterial numbers on or in carcase tissues. Choice of examination site influences the apparent extent of bacterial growth; the jowl, neck, brisket, shoulder, foreleg, breast flank, thigh, groin, diaphram and many other places can be selected for examination.

Levels of bacterial growth between sites can easily vary from abattoir to abattoir depending mainly on slaughter practices and season of the year[2]. Temperature of incubation plays a role in bacterial selection and in this manner influences the apparent pattern of bacterial growth on carcases. In broad terms all methods of estimating bacterial populations are approximate; especially in the presence of rapidly spreading bacteria such as *Proteus* and *Pseudomonas* spp. Methods requiring excision and homogenization of meat endeavour to ascertain the number of individual bacteria per gram of substrate. Results are largely dependant on the efficiency of homogenization. Scraping, swabbing and washing methods are employed to estimate the number of individual bacterial per cm^2 of surface. Results are influenced by the efficiency of removal and of course, by the subsequent degree of dispersion or homogenization attained. In methods dependent on dispersion and homogenization it is open to conjecture whether the count is of individual bacteria or of small groups of bacteria. Direct or indirect contact examination techniques are concerned with the number of bacterial micro-

colonies or colonies per cm² of surface. With regard to contact methods, which are of course, dependent on adhesive properties it is a matter of speculation whether representative bacteria are actually removed from all available clones. To related numbers of bacteria within a random group of colonies to the actual number of colonies is virtually impossible when it is remembered that a micro-colony, given the right conditions, can be quite a considerable macro-colony in the matter of 12–24 hours. Each method of determination has its place: individual bacterial numbers in terms of weight of bacterial growth; colony numbers with regard to extent of growth distribution.

Meat which has been in storage for some time may have been subjected to considerable handli
transportation and sharp variation in storage temperatures. Under such circumstances determination of the number of individual bacteria per gram of substrate or per cm² of surface may give the most useful index of bacterial growth. Obtaining the information is of course a laborious and time consuming exercise which tends, as a result of bacterial multiplication, to lose meaning with operational delays. In respect of slaughter technique and short term storage, contact and modified counting procedure determination of colony counts per cm² probably gives a better indication of the situation. Contact examination can be effected quickly and after the initial contact examination has been carried out, operational delays, within reason, are of limited consequence. In view of microscopic examination of colony numbers, results can be to hand within a compartively short time. In this respect therefore contact and modified counting procedure determination of colony counts per cm² may provide a useful screen to the need for determination of the number of individual bacterial per gram of substrate or per cm² of surface. Both direct and indirect contact examinations save time; direct contact examinations however, are less economical than indirect contact ones as the direct contact plates are more sophisticated, require more media per examination and preliminary pouring procedures are more meticulous. Modified counting procedures also save time. Fatigue and boredom are minimal and in this regard the 4 x HSC counting procedure is even more effective than the SSC procedure.

In view of the many factors requiring consideration, monitoring techniques over the six month trial period at Port Elizabeth abattoir, were limited to the indirect contact, warm carcase, 4 x HSC classification combination. This monitoring combination and the bar-graph arrangement of results were regarded as clearly defined operations, of a practical nature and of potential application in a generally complex situation.

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We are grateful for the help we have received at Grahamstown and Port Elizabeth abattoirs. We wish to thank all laboratory staff associated with the work, especially Misses M.J. Faye, W. Reyneke and A.P. Riches. We also wish to thank Dr. C.W.A. Belonje, particularly for administrative assistance and Dr. J.P. van der Merwe, Director of Veterinary Services, for permission to publish.

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BOOK REVIEW

BOEKRESENSIE

ANIMAL HEALTH YEARBOOK 1977 FAO – WHO – OIE

H.C. MUSSMAN, DIRECTOR, H. O KÖNIGSHÖFER, EDITOR
Animal Health Service, Animal Production and Health Division, FAO – 00100, Rome, Italy. PP 204 Maps 3

The Animal Health Yearbook compiled jointly by FAO, WHO and OIE is well known to veterinarians concerned with the infectious diseases of livestock.

It is a comprehensive record of the incidence of infectious diseases in eight regions of the world. In addition it contains statistical information on livestock populations and the number of veterinarians in various countries. It includes a comprehensive record of the type of foot-and-mouth-disease virus identified at the World Reference Laboratory for Foot-and-Mouth-Disease and gives data on the distribution as well as control measures employed in various regions of the world. Changes in the livestock disease position as well as a brief review on swine vesicular disease, brings the publication up to date. As a source of reference on the incidence of diseases, this publication is indispensable.

P.G.H.

CANINE ENCEPHALITOZOONOSIS IN SOUTH AFRICA

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

ABSTRACT: Botha W.S., Van Dellen A.F., Stewart C.G. **Canine encephalitozoonosis in South Africa.** *Journal of the South African Veterinary Association* (1979) **50** No. 2, 135 (En) Dept. Path., Fac. Vet. Science, Univ. Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

Clinical, clinicopathological, macroscopical and histopathological findings, as well as electron microscopic and cultural confirmation, in twelve natural cases of canine encephalitozoonosis are described, eleven of these under one year of age. Nervous symptoms, including blindness, retarded growth rate and nephritis are prominent clinical signs. The macroscopic findings and histopathology proved of great help in the diagnosis of the disease even in chronic cases. The organism was cultured and some distinguishing electron microscopical features between *Encephalitozoon* and *Nosema* are discussed.

A transmission experiment confirmed the diagnosis. The results are compared with the reported cases in dogs and Blue Foxes *(Alopex lagopus).*

INTRODUCTION

Canine encephalitozoonosis has been a diagnostic challenge. When the "nervous form of canine distemper" was described as early as 1923, "parasites" which were identical by light microscopy to *Encephalitozoon* were found in the brains of five out of twenty-two animals examined for distemper[7]. These "parasites" were thought to be related to distemper, although it was stated that it was incomprehensible that the "parasites" had not been seen previously[7]. No differentiation could be made between those dogs with "parasites" and those with canine distemper on grounds of symptomatology. Although these authors differentiated the organism from *Toxoplasma,* they did not identify it. In a detailed study of the pathology of canine distemper during 1930, a case with protozoan "parasites" in the brain was recorded[16]. These "parasites" were similar to those associated previously with spontaneous encephalitis of small rodents and rabbits. The parasite was identified in rabbits by Levaditi, Nicolau and Schoen and named *Encephalitozoon cuniculi* in 1923[9]. Nevertheless, the presence of the *Encephalitozoon* organisms in the case of canine distemper was believed to be accidental[16].

The disease continued to be a diagnostic challenge even after it was described as an encephalitis-nephritis syndrome of dogs, considered to be acquired as a congenital infection[19]. Plowright & Yeoman described the disease in two littermate puppies from East Africa in that same year[20]. Canine encephalitozoonosis was reported as nosematosis for the first time from the Republic of South Africa during 1966[3]. Since then nosematosis (encephalitozoonosis) has been described in this country in a Siamese kitten[26]. Three of its littermates showed nervous systems.

Canine encephalitozoonosis was brought to our attention as a problem in a breeding kennel where severe losses of unweaned puppies were encountered. The breeder complained that new owners returned pups to them with the belief that they were suffering from "distemper". When cared for intensively some recovered spontaneously. Since then similar cases have come to our attention.

This paper gives some of the clinical and clinicopathological findings with macroscopical and histopathological support of twelve natural cases of canine encephalitozoonosis. The organism was isolated in tissue culture from one animal. Electron microscopical evidence for the presence of the organism is given for some cases, and a transmission experiment was done for further confirmation. It is anticipated that these findings will be of some aid to clinicians and pathologists in the diagnosis of this disease.

MATERIALS AND METHODS

Twelve natural cases identified alphabetically (Table 1) and five experimental cases identified by numbering (Table 5) were studied.

Specimens were collected in 10 % formalin for histopathological examination. Sections were cut and stained with haematoxylin and eosin (HE) and Gram's stain (Brown-Hopps modification)[10]. On selected sections, Gomori's methanamine silver (GMS), periodic acid-Schiff (PAS), Giemsa and Ziehl-Neelsen (ZN) stains were used. Serial sections were cut of the brain of Case C and thirteen parts selected for examination. Four sections of the spinal cord of this animal were also cut. Only the kidney of Case L was examined.

One kidney was collected aseptically from Case A. Primary tissue cultures were prepared by trypsinisation. When the culture became confluent, subpassages were made into 100 mℓ flats and into test tubes containing cover slips. These cover slips were stained with Giemsa or Gram's stain and examined microscopically.

For transmission electron microscopy, organisms harvested from tissue cultures as well as brain and kidney from Cases A, D and E were used. These specimens were collected in 2 % Millonig's phosphate buffered glutaraldehyde (pH 7,35), postfixed in 2 % buffered osmium tetroxide, dehydrated in an ethanol gradient and embedded in Epon 812. Epon blocks were polymerised at 60 °C for 48 hours. Silver sections (60 μm) were cut on a Reichert OM U3 ultramicrotome, mounted on 300 mesh copper grids, and stained with 1 % aqueous uranyl acetate and 0,2 % lead citrate. Sections were examined with a Philips EM 301 electron microscope.

The tissue section lifting technique[24] for electron microscopy was employed to confirm encephalitozoonosis in Dogs L and 2. Blocks (1 mm thick) of formalin fixed tissue were cut from the kidney of dog L and flat-

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embedded in Epon. Sections 10 μm thick were cut by sliding microtomy. Specific focal lesions ± 0,1–1,0 mm in diameter were selected in the medulla and lifted from the 10 μm sections by means of the microlift.

The lifting technique was also employed to identify organisms found in lesions of Dog 2, but in this case the technique was applied to a 10 μ paraffin section which was deparaffinised and then infiltrated with Epon. Ultrathin sections were cut and stained using standard techniques.

Organisms from the supernatant of a primary culture were concentrated by centrifugation, dehydrated in an ethanol gradient and collected in amyl acetate. They were then layered onto a Nuclepore filter (0,1 μm pores) which was dried in a critical-point drier. The filters were mounted on stubs with conducting silver paint, vacuum-coated with carbon and gold (± 20 nm) and viewed in a Jeol JSMU 3 scanning electron microscope.

The transmission experiment was done at an early stage of the investigation, before culturing. Tissue material (brain, kidney, liver and spleen) was collected from natural Cases A to E and dosed to four healthy six-week-old Fox Terrier littermates, which were numbered 1 to 4. A fifth littermate (Dog 5) received eight mℓ of homogenised pooled kidney and brain material from dog B intraperitoneally. A mixture containing 600 mg procain penicillin and 750 mg dehydrostreptomycin* was added to the pooled tissue material. The experimental design is given in Table 5. All the experimental dogs were vaccinated against canine distemper, and 20 mg/kg methylprednisolone** was given 50 hours prior to infection and repeated twice at fortnightly intervals in an attempt to suppress natural immunity.

RESULTS

Clinical findings and clinical pathology

Details of the breed, sex and age of the twelve natural cases, as given in Table 1, show that the disease occurs in many breeds and both sexes. All animals except one were under nine months of age, which suggests that the disease occurred mainly in young dogs. Cases A to D are from the kennel where considerable problems with raising pups were encountered. The Rottweiler cases F to H were littermates from a single litter of ten pups of which only one survived. Case J was one of a litter of eleven Weimaraner pups of which nine had died during the preceeding three months. The Labrador, (pup L) was from a litter of three animals, all of which had showed nervous symptoms and had been euthanised owing to suspected distemper.

Table 1: DETAILS OF TWELVE NATURAL CASES

Case	Breed	Sex	Age (months)
A	Toy Pomeranian	male	1,5
B	Toy Pomeranian	male	1,5
C	Toy Pomeranian	female	2
D	Toy Pomeranian	male	2
E	Boerboel	female	2
F	Rottweiler	female	2
G	Rottweiler	female	3
H	Rottweiler	male	3
I	Schipperke	female	9
J	Weimaraner	female	5
K	Pointer	male	84
L	Labrador	male	3

Some of the more prominent clinical symptoms are given in Table 2. It is apparent that nervous symptoms occurred mainly in the younger age groups (cases A to F and L). Intermittent convulsions, unsteady gait, chorea-like spasms, hyperaesthesia, dysmetria and epileptiform fits were evidence of an acute meningoencephalitis in pups A, B and L. Incoordination and depression were seen in most pups younger than three months of age. The severity of ataxia varied noticeably. Blindness or partial blindness was intermittent and of amaurotic nature. Only dog F was reported to be aggressive and would immediately start biting when handled, which is consistent with the observations by Plowright & Yeoman[19]. Affected Toy Pomeranians gave periodic outbursts of low to high frequency howling.

Stunted growth occurred in most animals except in the very early stages of the disease. Pups C and D together had a mass of 339,6 g at two months of age compared to 1018,8 g for their two apparently normal littermates. Affected animals were apathetic (see Fig. 1), dehydrated and a mucopurulent conjunctivitis was present in some cases. Enlargement of the superficial lymph nodes was present in the subacute and chronic stage of the disease (Pups G, H, I and J). Clinical evidence of nephritis was also found during this stage of disease.The animals were emaciated and enlarged kidneys were easily palpable. The Schipperke of 9 months (case I) and Weimaraner of 5 months (case J) were admitted with a history of chronic relapsing nephritis.

The Pointer (case K) was the only adult dog with the disease and it had a history of periodic relapses to *Ehrlichia canis* infection for the preceding eighteen months. For this infection the dog was treated with oxy-

Table 2: PROMINENT CLINICAL SIGNS

Case	A	B	C	D	E	F	G	H	I	J	K	L
Convulsions	+++	++	–	–	–	–	–	–	–	–	–	+
Ataxia	++	+++	+	+	++	+	–	–	–	–	–	++
Depression	++	+++	++	++	++	++	–	–	+	+	+	+
Blindness	+	+	–	–	++	+++	–	–	–	–	–	–
Stunted	–	–	+++	+++	++	+	++	++	+	+	+++	–
Nephritis	–	–	–	–	+	++	–	++	+++	+++	–	–

\+ mild
++ moderate
+++ marked

*Streptopenicillin Vet Novo (Pty) Ltd.
**Depo-Medrol V Upjohn (Pty) Ltd.

Table 3: CLINICAL PATHOLOGICAL RESULTS

	Case	E	G	H	I	J	K	Normal*
A.	HAEMATOLOGY							
	Haemoglobin g/ℓ	114	122	110	–	67	101	120–180
	Red cell count 10^{12}/ℓ	5,0	2,95	3,04	–	2,39	3,10	5,5–8,5
	Haematocrit	0,39	0,24	0,25	–	0,20	0,25	0,37–0,55
	White cell count 10^{9}/ℓ	25,1	14,2	12,2	–	24,5	5,5	6–17
	Neutrophils	0,38	–	–	–	0,79	–	0,60–0,77
	Lymphocytes	0,56	–	–	–	0,08	–	0,12–0,30
	Monocytes	0,04	–	–	–	0,01	–	0,03–0,01
	Eosinophils	0,02	–	–	–	0	–	0,02–0,01
	Basophiles	0	–	–	–	0	–	rare
B.	BLOOD CHEMISTRY							
	BUN mmol/ℓ	1,8	1,4	4,6	14,0	33,0	4,0	2,5–4,2
	SGPT IU/ℓ	20	27	18	–	7	–	6–25
	SAP IU/ℓ	198	245	291	–	281	–	50–122
	TSP g/ℓ	85	56	77	–	109	–	67±6

*Normal values used by the clinical pathology laboratory in the Department of Medicine, Faculty of Veterinary Science, University of Pretoria

tetracycline* at a dose of 5 mg/kg body mass on several occasions and also with the ampicillin trihydrate** and stilboestrol dipropionate*** for prostatic hyperplasia. An apparent complete recovery was made but one month later the dog became anorectic, listless and soon developed a stunted, emaciated appearance. Severe thickening of both irises was noticed and on ophthalmoscopic examination marked dilatation of retinal vessels was observed. At no stage were any nervous or nephritic symptoms noticed in this animal.

Clinicopathological tests, as recorded in Table 3, were only carried out on some animals. In most instances a significant reduction in the haemglobin level, red cell count and haematocrit values were evident. All these cases had severe kidney involvement. The anaemia is therefore thought to be secondary and of aplastic origin owing to the renal disease. The leucocytosis of two dogs was due to different and inconclusive differential white cell counts. The leucopaenia in Dog K was probably due to the chronic ehrlichiosis. Mild to severe increases of the blood urea nitrogen (BUN) provided clinicopathological evidence of kidney damage in the chronic stage. Serum alkaline phosphatase (SAP) enzyme level was greatly increased in every animal tested. The significance of this is not known but it is postulated that it was associated with subclinical osteodystrophia fibrosa of renal origin. An increased total serum protein (TSP) was found in three out of four dogs tested and electrophoresis showed a hypergammaglobulinaemia in dog E.

Macroscopic Pathology

The carcases were in poor condition or even emaciated. The most significant gross lesions were usually in the kidneys. Mild, bilateral renal enlargement, pale brown colour and some petechial haemorrhages were encountered on the kidney cortical surfaces of dogs A to D. Dogs E to G had markedly swollen kidneys with focal, disseminated white spots of "pin-point" (± 1 mm) size were noticed on the surface. The capsule stripped freely, leaving a smooth, mottled and focally congested surface in case F. An irregular and granular kidney cortex was found in dogs E and G. These two dogs also had focal white spots and radiating white lines of less than 1 mm width on the cut renal surfaces from cortex to medulla. The kidneys of dogs H to J had a shrunken, pale grey and irregularly pitted cortical surface (See Fig. 3.). The capsule appeared white and thickened and cortical

*Liquamycin 100 V. Pfizer
**Penbritin V. Beecham
***Stilboestrol dipropionate V. Maybaker

Fig. 1. Toy Pomeranian C was stunted and had a head tilt to the left side.

Fig. 2. Thickening, irregularity and petecheal haemorrhages were visible on the irises of Dok K.

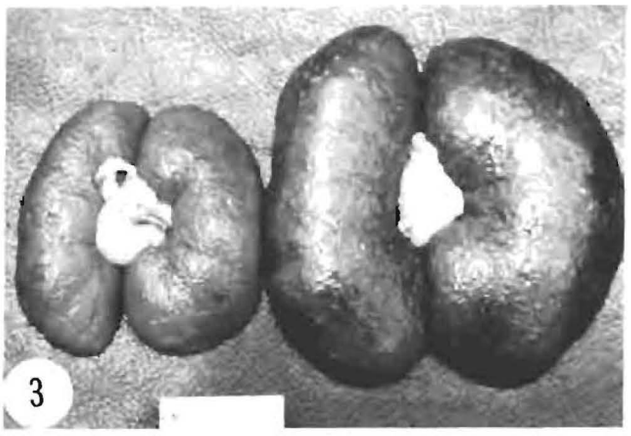


Fig. 3. The kidneys of two Rottweiler littermates G and H at three months of age. The small shrunken kidney of dog H is on the left.

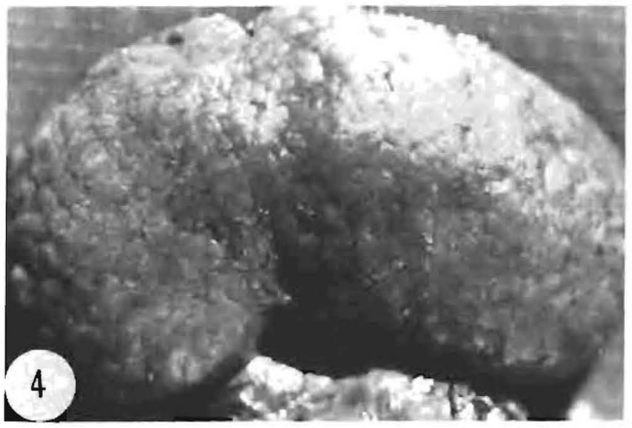


Fig. 4. The kidneys from Dog I had irregular pitted cortical surfaces.

tissue was torn away with stripping. Many retention cysts of varying size (0,5 to 3 mm) were present in the cortex. On sagittal incision the kidneys were tough and fibrotic. Irregular bands of fibrous tissue were present in the cortex and medulla. Cortical atrophy was most advanced in dogs I and J. Bilateral enlargement, irregular surfaces and focal disseminated white spots were recorded in the kidneys of dogs K and L.

A marked thickening with irregularities and focal white spots occurred in both irises of dog K (See Fig. 2.). Petechial haemorrhages were numerous on the irises and mild superficial corneal opacity was also present. No macroscopic ocular pathologic change was recorded in any of the young dogs.

The liver of dog E had many focal, irregularly distributed white spots of 1 to 2 mm diameter. These were visible underneath the capsule and present in the parenchyma on cut section.

A moderate splenomegaly and lymph node enlargement owing to cortical lymphoid hyperplasia were found in dogs G, H, I and K.

An osteodystrophia fibrosa which was thought to follow on the renal insufficiency and ureamia was found in dog J.

Dog I had a haemorrhagic cystitis characterized by a swollen mucous membrane, numerous petechial haemorrhages and severe congestion. This was not found in any of the other animals.

No macroscopically visible lesions of the brain or meninges were seen.

Histopathology

The most significant histopathological findings are given in Table 4. Meningitis was present in all cases examined. The leptomeninges were either focally or diffusely infiltrated by large and small mononuclear cells, including some plasma cells. The cellular infiltrates were more dense around vessels and in some instances involved their walls. Two distinct lesions, recorded as cuffing and microgranulomas, typified the encephalitis. In Dogs A to C the perivascular cuffing was mild and consisted largely of hyperplastic pericytes. In the other dogs (except Dog H) a moderate to severe granulomatous perivasculitis was found which consisted of mononuclear cells, including numerous plasma cells. A mild encephalitis was found in Dog H.

The characteristic microgranulomas were focal areas with increased cellular density and included epithelioid cells, macrophages and other mononuclear cells such as lymphocytes and plasma cells (Fig. 5.). Some microgranulomas were associated with capillaries but the presence of blood vessels could not be confirmed in the majority of cases. It appeared that both the astrocytes and microglia had undergone hypertrophy and hyperplasia to contribute towards the formation of these microgranulomas. Epithelioid cells, macrophages and other mononuclear cells were present as well. A mild Nissl degeneration of neurons and status spongiosus was found in association with these lesions. Microgranulomas were numerous even in the very young dogs (Cases A to F) and were present also in older animals. The neuropathological lesions were distributed at all levels of the brain and spinal cord that were examined, and no predilection site was apparent.

A moderate to severe interstitial nephritis was present in all but the very young dogs. In puppies A to D the acute nephritis was characterised by a histiocytic and epithelioid cellular reaction which was located mainly in the medullary rays and at the corticomedullary junctions. In case E it was estimated that approximately 40 % of the cortex was replaced or displaced by cellular infiltrates. These lesions had a linear distribution along tubules, and consisted of necrotic tubular epithelial cells, histiocytes and epithelioid cells surrounded by mononuclear infiltrates. A severe, acute interstitial nephritis with lymphocyte, plasma cell, macrophage and neutrophil infiltration was found in the Rottweilers F and G.

The kidneys of animals H and L showed the commencement of fibroplasia and a relative increase in the number of plasma cells. Many hyalin casts were present in the collecting tubules. Severe, chronic interstitial nephritis was present in Dogs I, J and K. This was recognised by the mature collagen in the fibrous bands attached to the capsule and surrounding tubules in the medulla. This fibroplasia caused tubular obstruction and dilatation. Retention cysts were numerous. Plasma cells were the only other inflammatory cells present in these chronic cases. Bowman's capsules were thickened and glomeruli were atrophied. Some hyalin casts stained blue with HE owing to the presence of mineral salts. Metastatic calcification was obvious in the kidney of dog I. An acute pyelonephritis and haemorrhagic cystitis was also present in this animal.

Multifocal, granulomatous hephatitis consisting of focal areas of perivascular mononuclear infiltration and microgranulomas were noticed in six animals. Microgranulomas involving three to four hepatocytic cords

Table 4: HISTOPATHOLOGY

Case	A	B	C	D	E	F	G	H	I	J	K	L*
Meningitis focal		+					++	+	++			
diffuse	++		++	+	+++	++				+	++	
Encephalitis cuffing	+	+	+	++	+++	++	+++	+	+++	++	+++	
microgranulomas	+++	+++	+++	+++	+++	+++	++	+	++	+	++	
Nephritis acute	+	+	+	++	+++	+++	+++					
subacute								++				++
chronic									+++	+++	+++	
Hepatitis	++	++	–	+	+++	+	++	+	+	+	++	
Myocarditis	–	+	–	+	+	–	–	–	–	–	–	
Pneumonitis	++	–	–	+	+++	–	++	+	–	–	–	
Organisms present	n	n	n	n	f	f	f	f	f	f	f	n

+ mild
++ moderate
+++ marked

n numerous
f few
* Only kidney examined

were found in dog E (Fig. 8.). A mild portal lymphocytic reaction was present in all cases involved. The microgranulomas were distributed throughout the liver lobule.

Mild, focal myocarditis was found in three dogs. The cellular reaction was similar to that of the hepatic microgranulomas, and organisms were demonstrated within inflammatory foci of two cases.

Pneumonitis, observed as thickening of alveolar walls owing to fibrinous exudation and proliferation of alveolar epithelial cells, was present in five animals (Fig. 7.). In Dog E the pneumonitis was prominent with an occasional lymphocytic and plasmacytic focus.

Two dogs (Cases E and K) had a marked lymphocytic and plasmacytic infiltration in the iris. Mild perivascular plasma cell infiltration was present in the retina, sclera and optic nerve. Organisms were demonstrated in the irises of both cases.

Segmental vasculitis was noticed in Case I as an acute fibrinoid necrosis of the wall of small to medium cerebral arteries but was more commonly characterised as infiltration of lymphocytes and plasma cells into the vessel wall (Fig. 6.).

Encephalitozoon organisms were recognized by their morphology and specific histochemical staining reaction. Spores measured on the average 1.5 x 2.5 μm (therefore smaller than *Toxoplasma*) and some organisms contained a typical polar vacuole (unstained spot) in the cytoplasm. The organism stained Gram-positive, acid-fast and some had a PAS- and GMS-positive granule in the apparent anterior pole. Dog L

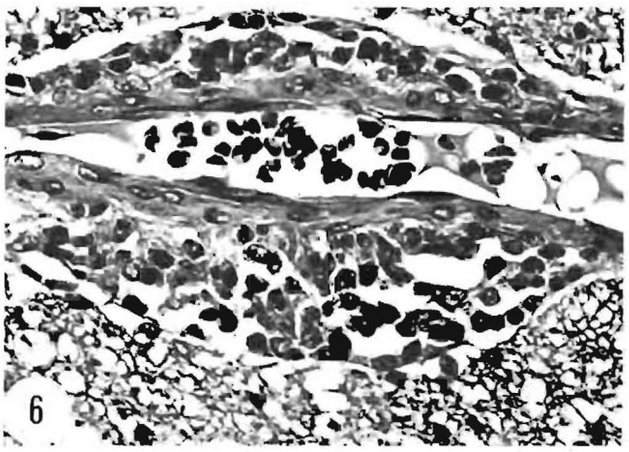


Fig. 6. Segmental vasculitis and perivascular cuffing in the brain of Dog I. HE x 640.

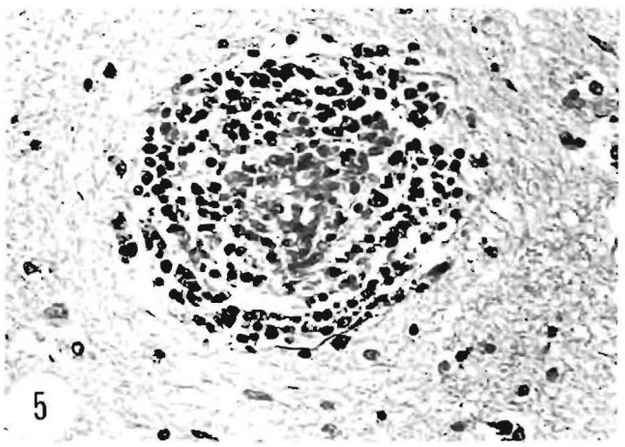


Fig. 5. A microgranuloma in the brain of Dog K. HE x 400.

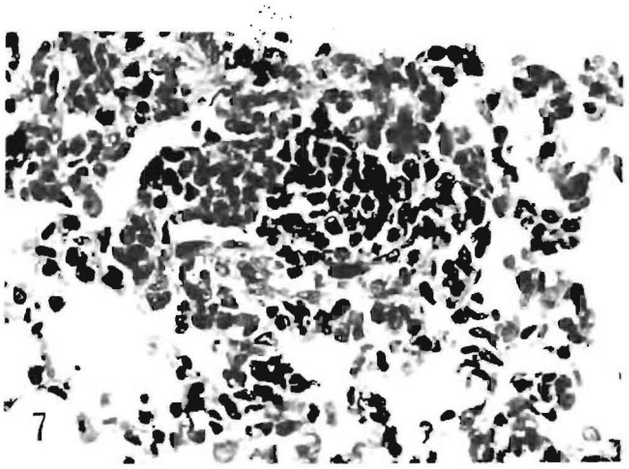


Fig. 7. Focal pneumonitis in the lung of Dog H. HE x 400.

had numerous PAS- and GMS-positive spores in pericytes and macrophages in the interstitial tissue of the renal medulla. Cyst-like groups contained a variable number of spores which were usually intracellular in endothelial cells or in macrophages along capillaries. These cyst-like groups were demonstrated in the brain, kidney, liver, lung, myocardium, eye and spleen. In some intances they occurred within the microgranulomas.

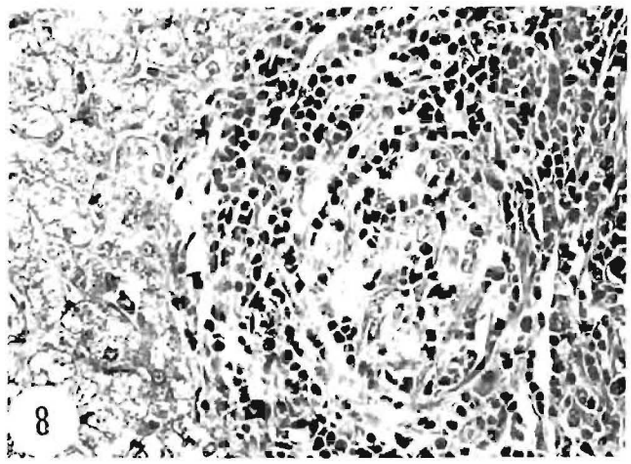


Fig. 8. A hepatic granuloma in dog E. HE x 400.

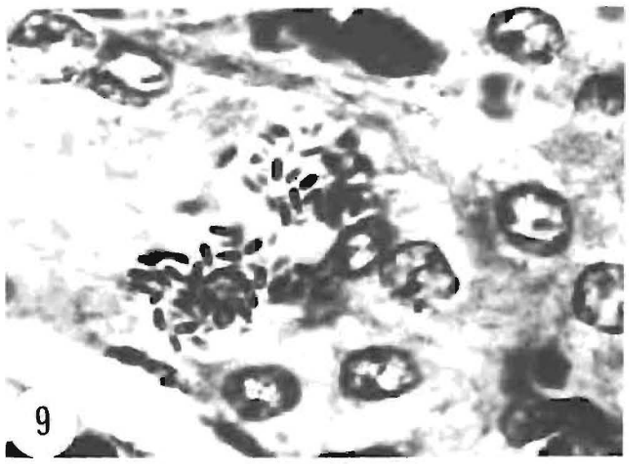


Fig. 9. *Encephalitozoon* organisms in kidney tubule cells of Dog 2. Note the polar vacuole in some organisms Gram x 1200.

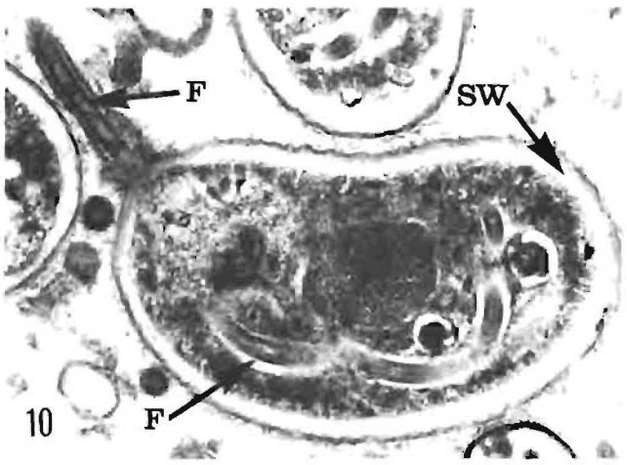


Fig. 10. An electron micrograph of the *Encephalitozoon* organisms from tissue culture. Note the partially everted filament (F) and spore wall (SW) x 24500.

Culturing

During the second subpassage of the tissue culture prepared from dog A, a protozoan-like parasite was seen in vacuoles within some of the cells. These organisms were morphologically identical to *Encephalitozoon*[9 18]. Groups of spores were seen within the vacuole of infected cells. The individual spores were slightly avoid with rounded ends. They did not stain well with Giemsa but were Gram-positive. Details of the isolation will be described separately[23].

Electron Microscopy

The aetiologic agent, *Encephalitozoon,* was studied and confirmed in lesions of the brain and kidneys of dogs A, D, E and I. Intracellular proliferative forms, sporonts, sporoblasts and spores were observed (Fig. 11.). A single nucleus was present in all stages of the reproductive life cycle, and typical organellae, including filament, polaroplast and ribosome-like structures, were seen in the spore.

Microgranulomas in the liver of Dog E were selected for examination by lifting them from formalin-fixed, Epon-embedded sections. These were shown to contain degenerated spores in which no recognisable organellae had remained (Fig. 13.).

Gram-stained sections of paraffin-embedded tissue from Dog 2 were re-embedded in epon, and suspected organisms lifted from a kidney collecting tubule by the lifting technique[24]. Though considerable ultrastructural detail was lost, sufficient structural detail of organellae such as the filament was preserved and demonstrated to permit identification of the organisms as microsporida.

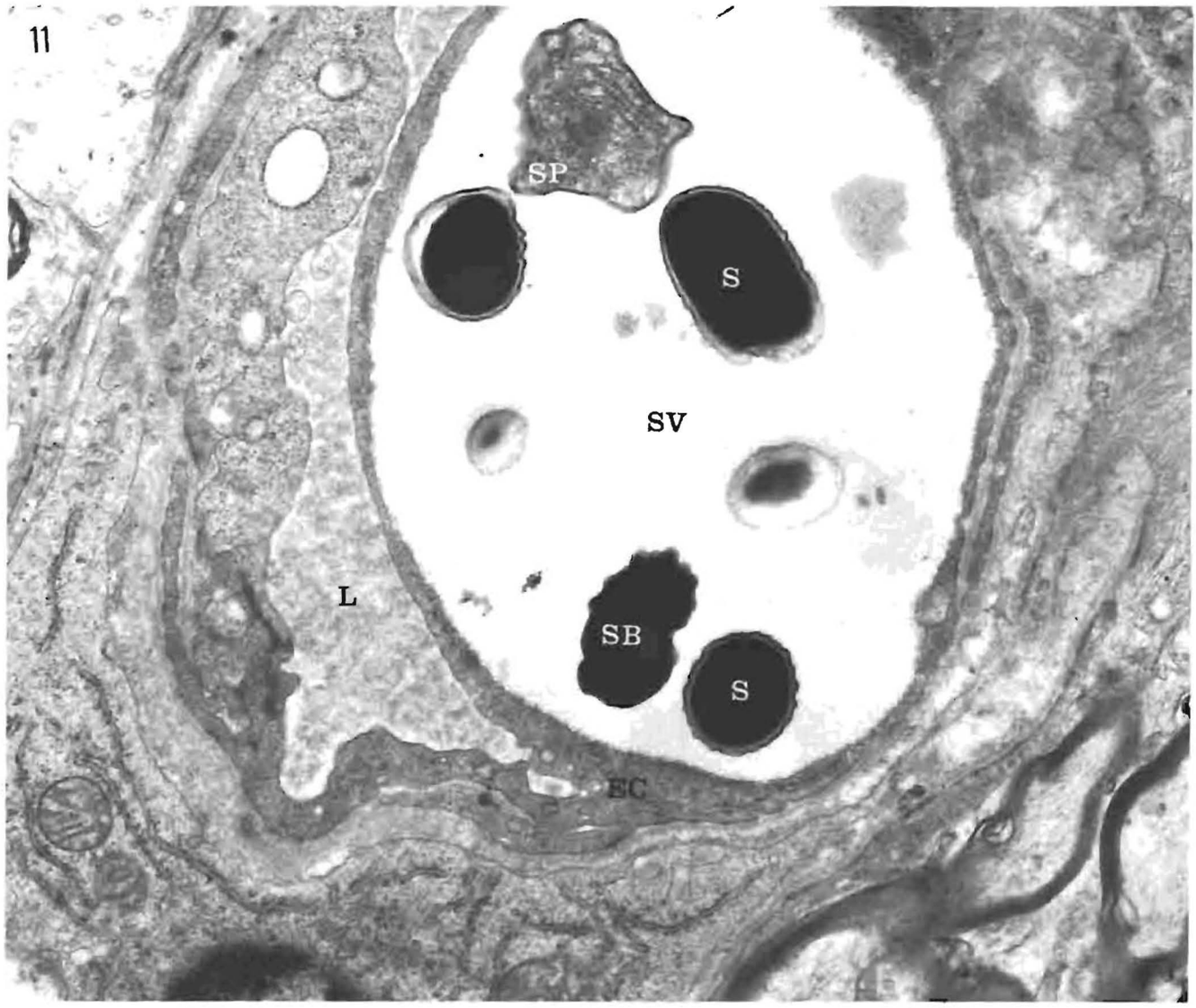
During examination of parasitised tissue culture cells, sporoblasts and spores were found free in the sporogony vacuole, while developing sporonts were still attached to the cytoplasm of the host cell (Fig. 12.). The ejected filament could be demonstrated with apparent partial or complete emptying of the spore content (See Fig. 10.).

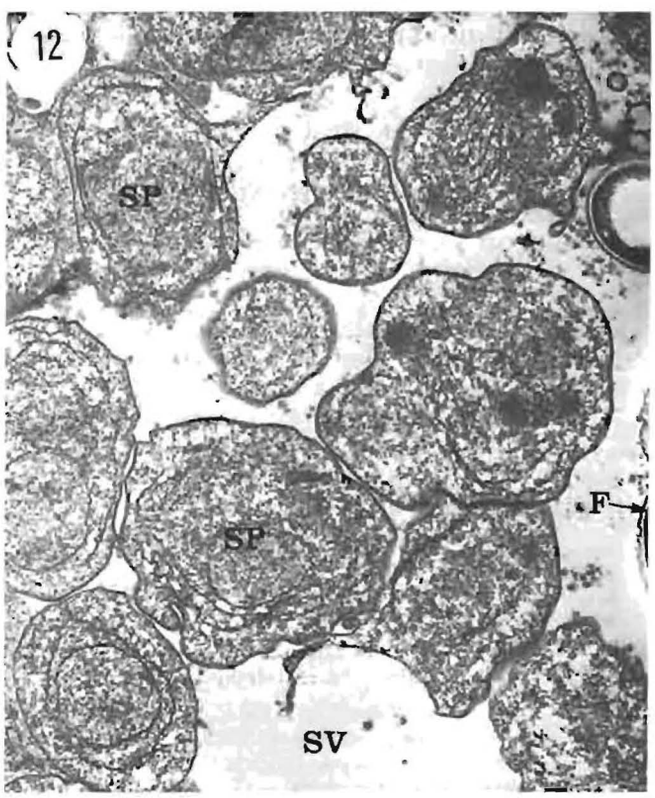
Scanning electron-micrography showed that the *Encephalitozoon* organism had a smooth surface. The size varied from 2,75 to 2,5 μm in length by 1,08 to 1,42 μm in width. The length of most of the organisms was, however, very close to 2,75 μm. In some instances the organism appeared "kidney shaped" (See. Fig. 14.). Folding of the spore wall was noticed with expulsion of the filament in one instance. (See Fig. 15.)

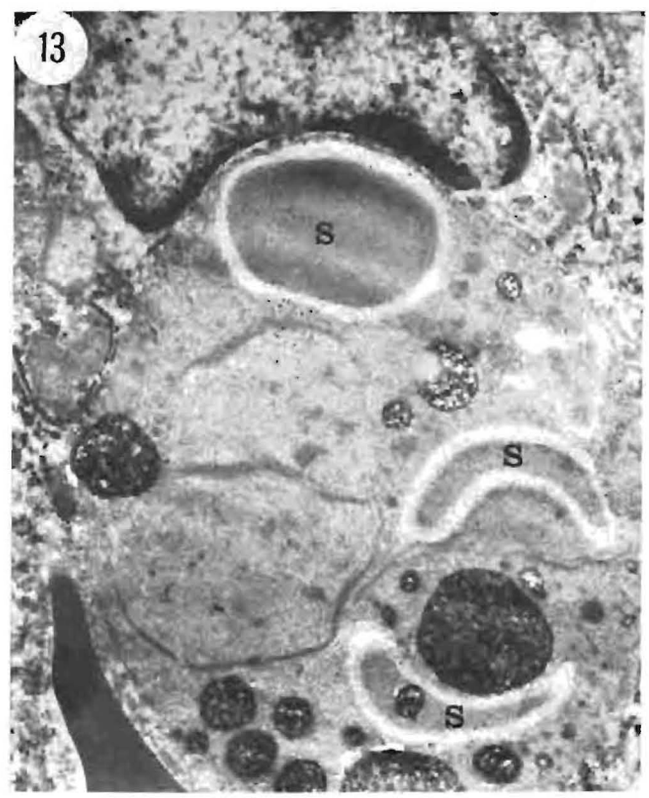
Fig. 11. A parasitised endothelial cell (EC) and partial occlusion of the capillary lumen (L) from the brain of Dog E. Sporont (SP), sporoblast (SB) and spores (S) are present in the sporogony vacuole (SV). x 1800.

Fig. 12. The sporogony vacuole (SV) of an infected tissue culture cell contains some sporonts (SP). The filament (F) is visible in cross section of a spore. x 13500.

Fig. 13. Three degenerated spores (S) are seen in the cytoplasm of a macrophage from the livergranuloma of Dog E. x 16400.







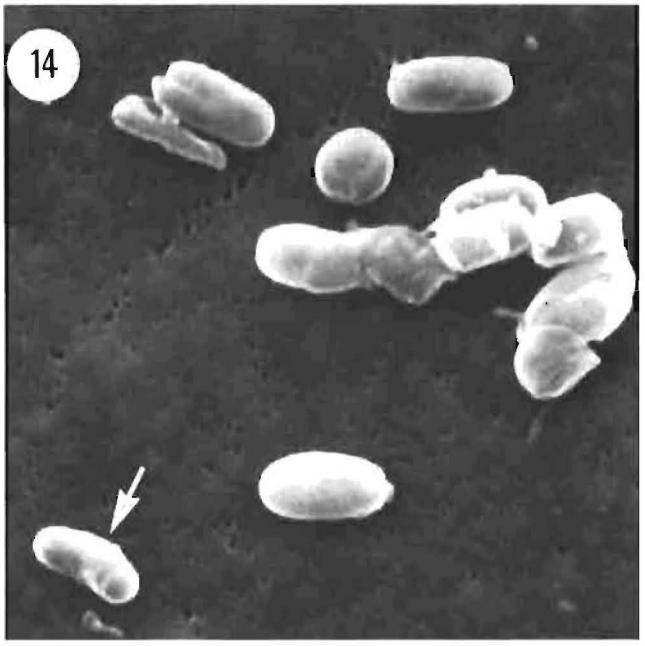


Fig. 14. Scanning electron micrograph of *Encephalitozoon* organisms from tissue culture. A "kidney shaped" spore is indicated by the arrow. x 6000.

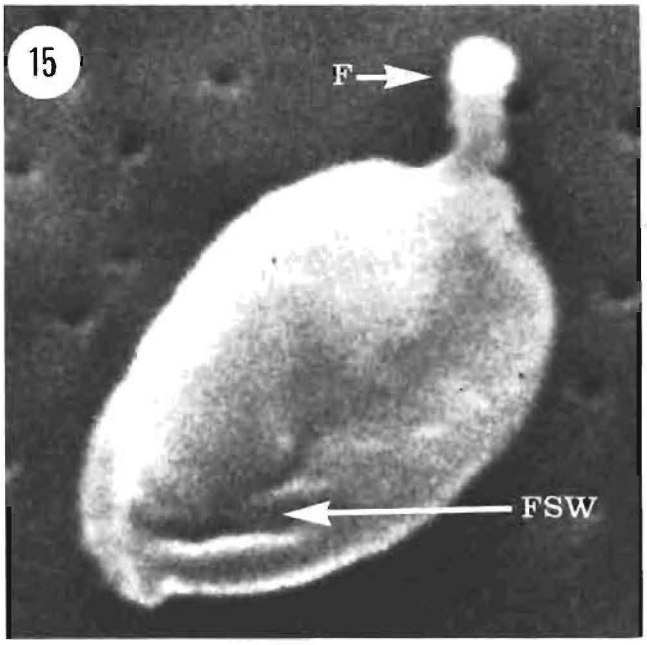


Fig. 15. Expulsion of the filament (F) which contained a slightly enlarged anterior end is seen in this spore. Note the folding of the spore wall (FSW) x 33000.

Transmission study

The disease was transmitted orally in four out of four dogs using suspected infective material from naturally diseased dogs A to E,

Dogs 1 to 4 (see Table 5) developed no clinical symptoms comparable to the natural disease except that they appeared stunted. The experimental animals were euthanised after two to three months. Multifocal white spots (± 1 to 2 mm in diameter) were found on the surfaces and cut sections of the kidneys in Dogs 2, 3 and 4. Their kidneys also appeared swollen. A focal disseminated pneumonitis was present in Dog 4.

Dog 5, that had received 8 mℓ of pooled brain and kidney material by intraperitoneal injection, died within 15 minutes apparently owing to shock. Examination of this animal showed no specific macroscopic or histopathologic lesions.

Upon histopathological examination the neuropathological changes were seen to be similar to that found in the natural disease but to a milder degree. A very mild, focal meningitis, noticed by the infiltration of a few mononuclear cells into the pia mater, was present in Dogs 1 and 2. The focal granulomatous encephalitis was regarded as minimal in Dog 3 and mild in the other animals. This consisted of multifocal microgranulomas scattered throughout gray and white matter. A diffuse gliosis was noticed in the cerebrum of Dog 4. Segmental vasculitis was found in the brain of Dog 2.

A mild to moderate multifocal interstitial nephritis was a prominent finding in all four animals that had received infective material orally. Focal areas of lymphocytic infiltration were apparently most numerous and largest at the corticomedullary junctions. Most of the inflammatory foci had a linear and radially arranged pattern. In one instance, (Dog 3) had a linear lesion involving a medullary ray from the cortex corticis into the outer medulla.

A mild granulomatous hepatitis found in three dogs consisted of small, focal, disseminated areas of mononuclear cell infiltration together with Kupffer cell hyperplasia. The Kupffer cells appeared hypertrophic and prominent in most sinusoids. A moderate focal pneumonitis was found in Dog 4.

Encephalitozoon organisms were demonstrated in tissue sections of Dogs 1 to 4 using Gram's, PAS, GMS and ZN stains. They were not numerous but were often associated with the granulomatous inflammation. Organisms were also seen in tubule epithelial cells where they initiated no cellular reaction (See Fig. 9.). The shape, size and staining characteristics were consistent with those of *Encephalitozoon*. Inadequate fixation for electron microscopy made it impossible to confirm the genus of the microsporidan parasite in the lifted sections from the kidney of Dog 2.

Table 5: EXPERIMENTAL DESIGN AND PATHOLOGY: TRANSMISSION STUDY

Dog Number	1	2	3	4	5
INFECTIVE MATERIAL					
origin*	B	ACD	BDE	BDE	B
tissue type	brain kidney	kidney	liver spleen	brain	brain kidney
route	oral	oral	oral	oral	ip
INTERVAL***	2	2,5	2,5	3	**
CLINICAL	na	na	na	na	shock
MACROSCOPIC					
nephritis	–	+	++	++	–
pneumonitis	–	–	–	+	–
HISTOPATHOLOGIC					
meningitis	+	+	–	–	–
encephalitis	+	+	+	+	–
nephritis	+	+	++	++	–
hepatitis	+	+	–	+	–
myocarditis	–	–	–	–	–
pneumonitis	–	–	–	++	–
organisms	p	p	p	p	–

\+ mild ip intraperitoneal * See Table 1
++ moderate p present ** Died within 15 minutes
+++ marked na no abnormality *** months

DISCUSSION

It is believed that the data presented gives additional unequivocal evidence of the presence of canine encephalitozoonosis in South Africa. The relative importance of this disease is still to be determined, but it should be considered especially in the differential diagnosis of neurological and renal diseases in young dogs. In Europe, encephalitozoonosis has also been frequently found as a disease among cubs of the Norwegian Blue Fox *(Alopex lagopus)* [1 14 15]. Regular, yearly epizootics have been reported to occur in suricates (syn mierkat) *(Suricata suricatta)* while sporadic cases were found in clouded leopards *(Neofelis nebulosa)* and Blue Foxes *(Alopex lagopus)* in the Prague Zoo[27]. Encephalitozoon has been described as an occult endemic disease of the central nervous system of mice[6] and is known to occur in other laboratory animals [8 9 11 17 18]. A natural case of disseminated disease has been found in a cat[26]. An organism considered to be *Encephalitozoon cuniculi* was demonstrated in the cornea after superficial keratectomy in a domestic short-haired cat[4], but the disease is apparently relatively uncommon in this species.

A microsporidan parasite, found in an immunologically compromised infant was confirmed to be a *Nosema* sp[21]. An *Encephalitozoon* had been isolated from a human patient with encephalitic disease[12]. Microsporidans should therefore be considered as potential agents of zoonotic disease.

Clinical symptoms, observed in our series, did not include the "characteristic" spasmodic eye movements described by Plowright[19] and showed to some extent the variability of symptoms that could be expected. Not only can the disease be confused with distemper as reported [7 16] but the "fading puppy" syndrome, canine herpes infection, infectious canine hepatitis, toxoplasmosis, rabies, epilepsy, meningitis, encephalitis, otitis media, etc., may cause confusion. The weakness and incoordination of the hind quarters reported by Plowright[19] as being a "most noticeable feature" is difficult to differentiate from general weakness and unsteadiness.

Canine encephalitozoonosis is most probably transmitted transplacentally or neonatally and the disease is therefore prevalent in young dogs [3 13 19 20 25]. Transplacental transmission was also the way in which Blue Fox cubs were thought to be infected[14 15]. Neither in dogs[20], nor in Blue Foxes[15] did the parent animals show any symptoms of encephalitozoonosis.

Young dogs presented to the clinician with acute, subactue or chronic nephritis could be suffering from encephalitozoonosis, especially if there are nervous symptoms present or when a history of such symptoms is given. Renal biopsies may be useful in confirming the diagnosis.

Blindness or partial blindness, bilateral thickening of the irises and dilation of retinal vessels were found in our series. Ocular changes also include superficial and deep keratitis[2 3 4], retinitis[1 17] and cataracts[1].

Hypergammaglobinaemia, found in the dog of the present series, was also reported as a constant clinicopathological finding in severely affected Blue Foxes[14]. The reduced haemoglobin level, red cell count and haematocrit was thought to follow depressed erythrogenesis owing to the nephritis of encephalitozoonosis.

Dog K had a leucopaenia which could be expected in an animal suffering from ehrlichiosis. This lowered body resistence or immunoincompetance may have rendered this animal more susceptible to encephalitozoonosis. A clinical method for demonstrating organisms was described in rabbits[5]. Urine smears stained with Gram's stain (Brown-Hopps modification) were useful for screening, while Ziehl-Neelsen, acid-fast stain, using concentrate formaldehyde as a decolouriser, was found best for identification[5 10]. Indian-ink immunoreaction and indirect fluorescent antibody tests are described for rapid diagnosis of lapine encephalitozoonosis[28], and promise to be very useful diagnostic means in the canine disease.

The most commonly recorded autopsy findings in our series are changes associated with acute to chronic interstitial nephritis. Thrombosis of meningeal vessels and haemorrhagic encephalomalacic areas in the brain had been reported in one dog[3]. Blue Foxes found to survive for 6 months until pelting time, had greyish-white spots in the coronary grooves and liver[15]. The finding of ocular pathologic changes, focal disseminated hepatitis, pneumonitis, splenomegaly and lymph node enlargement may be of some aid in the diagnosis.

Histopathology is by far the most commonly used procedure by which a diagnosis is made[3 8 11 12 15 17 19 20 26 27]. In Blue Foxes, polyarteritis nodosa was found in many organs including the eye and it was suggested that a hypersensitivity reaction is provoked[1 15]. Vasculitis was suggested to be the underlying pathogenesis of the lesions in canine encephalitozoonosis[13 25]. The histopathologist must differentiate the *Encephalitozoon* organism from other protozoa such as *Nosema, Toxoplasma,* leishmanial forms of *Trypanosoma cruzi, Hepatozoon, Leishmania* and *Sarcocystis.* Many differentiating features are known[13 17 18 22 25]. Histopathological examination of brain material proved to be very valuable in cases of chronic interstitial nephritis (as in Cases I, J and K) caused by *Encephalitozoon,* because of the presence of meningoencephalitis and typical microgranulomas with organisms. In chronic cases the histopathological renal lesion is non-specific.

Culturing and electron microscopy are highly sophisticated diagnostic techniques which may add final proof of the identity of the organism. The tissue section lifting technique[24] for electron microscopy proved a very useful method for demonstration of the organism. Ultrastructural evidence is necessary to differentiate between the two microsporidan parasites *Encephalitozoon* and *Nosema*[13 22 25]. Recent research findings indicate that previously reported *"Nosema"* organisms in dogs are apparently all *Encephalitozoon* spp.[13 22]. Morphologic features such as single nuclei during all stages of the reproductive life cycle, size, shape and presence of the polar filament have been found in the organism that was isolated from dog A. These findings are consistent with those reported for *Encephalitozoon*[22].

The transmission experiment showed that oral infection is possible in dogs. Pooled infected kidney, brain, and a mixture of liver and spleen caused disease in the recipients. The apparent numerous lesions at the corticomedullary junctions of the kidney, diffuse organ involvement, vasculitis and presence of organisms in the vascular endothelium or pericytes suggest a primary haematogenous spread of organisms. The linear distribution of the inflammation along some tubules in the medullary rays of the kidney and the presence of organisms within the tubular lumen is indicative of intratu-

bular spread. The most probable way of natural infection is via the urine.

Canine encephalitozoonosis has been confused with canine distemper in the past[7] [16] and this undoubtedly happens today. Careful evaluation of the clinical signs, clinical pathology, gross and histopathology together with culturing, electron microsopy and the possibility of serological tests should aid to obtain a definative diagnosis.

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