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TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

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ARTICLE ARTIKEL

EVALUATION OF KIDNEY FUNCTION IN DOGS SUFFERING FROM CANINE ENCEPHALITOZOONOSIS BY STANDARD CLINICAL PATHOLOGICAL AND RADIOPHARMACEUTICAL TECHNIQUES

W.S BOTHA*, IRENE C. DORMEHL** and D.J. GOOSEN***

ABSTRACT: Botha W.S.; Dormehl Irene C.; Goosen D.J. **Evaluation of kidney function in dogs suffering from canine encephalitozoonosis by standard clinical pathological and radiopharmaceutical techniques.** *Journal of the South African Veterinary Association* (1986) **57** No. 2, 79-86 (En). P.O. Box 12731, 0110 Onderstepoort, Republic of South Africa.

Canine encephalitozoonosis can be responsible for a severe renal disease in dogs which may develop into progressive, irreversible kidney failure. Three pure-bred Boxer littermates with confirmed encephalitozoonosis were subjected to sequential clinical pathological tests and renal biopsies. The endogenous serum creatinine and urea levels showed an initial temporary reduction but later increased steadily.

The phenolsulphonphthalein retention test confirmed this end-stage renal disease. Initial hyper-gamma globulinaemia showed a rapid decline. Urinalysis was an indicator of chronic renal disease and the kidney biopsies confirmed progressive irreversible kidney lesions. Evaluation of sequential tests are advocated for the setting of a prognosis.

The radiopharmaceutical techniques employed proved to be sensitive indicators of renal dysfunction and a means of evaluating the function of the left and right kidney separately.

Key words: Encephalitozoonosis, canine, chronic renal failure, chronic nephritis, radiopharmaceuticals.

INTRODUCTION

When canine encephalitozoonosis was originally described by Plowright in 1952 it was named the "encephalitis-nephritis" syndrome[13]. Subsequent reports confirmed that kidney pathology is a common finding in this disease[1]. Renal involvement was also observed in blue foxes, cats, rabbits and mice suffering from this condition[10 12 13 17 18]. Recent studies showed that this disease may give rise to chronic interstitial nephritis and glomerulonepheritis[1 9 10]. It appears that the renal lesions may become irreversible in time and give rise to uraemia and death in some of the affected animals[1 15].

Chronic interstitial nephritis caused by canine encephalitozoonosis may be clinically occult for a long time and recognition of an infected dog at this subclinical stage is difficult[1]. Evaluation of kidney function is of importance as a prognostic measure. This study was conducted to evaluate some of the parameters used to determine kidney function during the chronic phase of renal disease due to encephalitozoonosis.

Lourens et al.[7] studied the application of radiopharmaceutical renogram techniques in dogs and found it to be a useful aid in evaluating kidney function and morphology.

MATERIALS AND METHODS

Three pure bred Boxer dogs were obtained from a breeder where the "fading puppy syndrome" was a problem and canine encephalitozoonosis was subsequently diagnosed. Two litter mates died at 4 and 8 weeks of age, respectively, showing nervous signs and in both cases encephalitozoonosis was confirmed by histopathology. The 3 live dogs were in poor condition, showed occasional aggressiveness and jerking spasms of the legs. Indirect immunofluorescent antibody titres for encephalitozoonosis were high in all three dogs and remained high after retesting 4 weeks later. The bitch showed an antibody titre of 1/160.

The dogs were numbered 80, 81 and 82, respectively. The mass of these dogs was determined monthly and compared with a control that was compiled determining the mass of 6 male and 6 female normal Boxer dogs at monthly intervals from 3 to 12 months of age on 2 different breeding premises.

Blood was collected at monthly intervals from Dogs 80, 81 and 82 for the determination of serum creatinine and urea levels using the standard methods of Popper et al.[14] and Gutman & Bergmeyer[3], respectively.

Total serum protein (TSP) was determined using a refractometer (TS meter, American Opical Co.) while quantitative electrophoresis was performed on cellulose-acetate membranes in a Beckman Microzone tank according to the method of Peck[11]. Serum from 3 clinically healthy dogs was also obtained for control purposes for each of the following age groups: 3, 5, 10 and 12 months.

The Phenolsulphonphthalien (PSP) retention test was performed at monthly intervals on Dogs 80, 81 and 82. PSP was injected intravenously at a dose rate of 1 mg/kg bodymass after a 4 ml blank heparinized blood sample was collected. Exactly 60 min after injecting the PSP, a second 4 ml heparinized blood sample was collected and the plasma used for colorimetric determination of the PSP present[5].

Standard urinalysis methods (Section of Chemical Pathology and Haematology, Faculty of Veterinary Science, Department of Medicine, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort) were used on the different urine specimens that were obtained by abdominal pressure. The Sternheimer-Malbin stain (Sternheimer & Malbin)[16] was used to stain the urine sediment for cytological purposes.

Kidney biopsies were obtained from Dogs 80, 81 and 82 at 5 and 7 months of age, from the left and right kidney, respectively. After general anaesthesia and

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preparation of the skin for sterile surgery, a 3 cm incision was made into the lateral abdominal wall in the paralumbar fossa. The incision penetrated the peritoneum to allow visual examination of both kidneys. For the purpose of obtaining a biopsy specimen, the kidney was fixed with one finger while a Tru-cut needle (Travenol Laboratories) was introduced through the incision and into the kidney. The needle biopsies were fixed in 10% buffered formalin and processed routinely for light microscopy (Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort). Masson's trichrome and Gram's (Brown-Hopps modification) stains were also used for staining of sections[8].

Radiorenograms were obtained using (99m) Tc-diethyltriamine-penta acetic acid (DTPA). This radiopharmaceutical is known to be predominantly excreted by the renal glomeruli[2]. For each isotope study the dogs were positioned on their stomachs to allow the crystal of the large-field gamma camera (ON 410 Sigma) to view both kidneys simultaneously from a dorsal projection. The dogs, which were conscious throughout the study, were injected intravenously with 500 μCi (18,5 MBq) of DTPA in the form of a bolus of 1 ml[7].

Acquisition of data for the renogram with the aid of a dataprocessor (A2-MDS), commenced on injection of the isotope. A series of 120 images were recorded at a rate of 10(s/)frame for each experimental run. In this manner a 20 min recording could be obtained of the passage of the radiopharmaceutical through the kidneys. The movement over time of radioactivity through the kidneys was evaluated by means of the facility of region of interest selection, of the computer software; time-activity curves were generated after the appropriate background correction in each case[2,4]. A second DTPA study was performed on each dog after 3 months.

An additional series of isotope studies was also performed on each dog using (99m) Tc-Dimethylsuccinic acid (DMSA) in order to evaluate the morphology of the kidneys and possible changes that might have occurred after a time interval of 3 months[6]. One week after the DTPA-study, each dog was injected intravenously with 500 μCi (18,5 MBq) of DMSA and scanned 30 min later, with the same positioning with respect to the crystal as before. The results were compiled after an optical evalution of each scan. The second series of DSMA scans were performed 3 months after the first set.

RESULTS

A. Mass

The mass of the different animals are presented in Fig. 1.

B. Serum creatinine, urea and PSP retention values

The results obtained from the different biochemical and PSP retention tests are given in Table 1.

C. Total serum proteins and electrophoresis

Results of the TSP and electrophoresis from Dogs 80, 81 and 82 as well as the different mean values of the control animals, are given in Table 2. Selected graphs from Dog 80 are given in Fig. 2 – 4. Note the marked

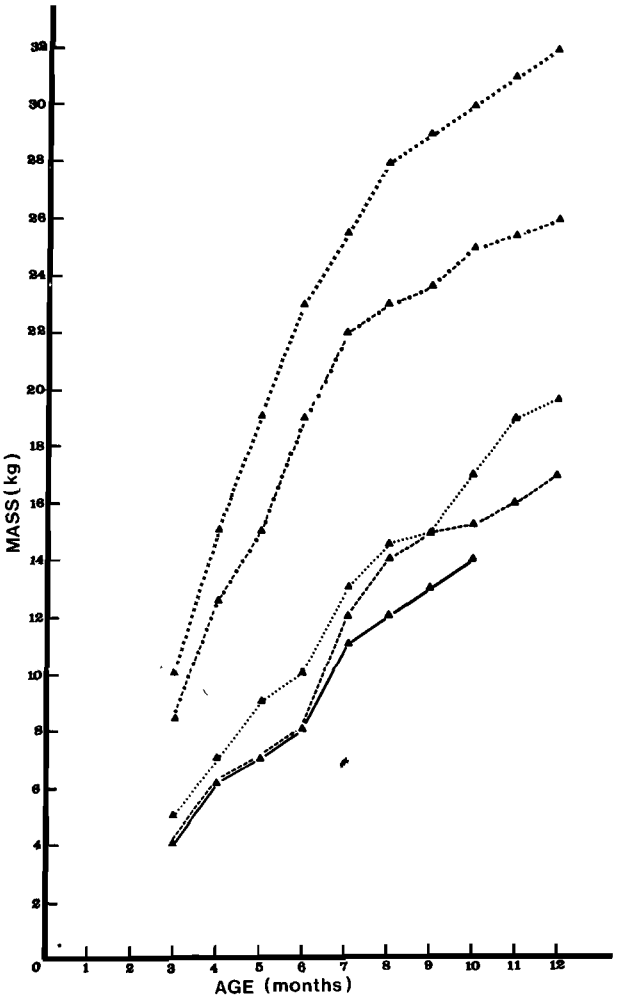


Fig. 1: The mass of control dogs is compared to the 3 diseased animals
Mean male controls ******* Dog 80 (female) .....
Mean female controls ._._._ Dog 81 (male) ----------
Dog 82 (female) _____

decrease in the gamma globulin fraction of the serum proteins (Fig. 2 – 4).

D. Urinalysis

The urinalysis results are given in Table 3.

E. Biopsies

When the abdomen was opened in the 5 month old dogs, the kidneys revealed bilaterally irregular surfaces with white foci (1 mm in diameter) and multiple clear retention cysts (1 – 2 mm in diameter) bulging from underneath the capsule. Even more irregular surfaces due to fibrous displacement of cortical tissue were found at 7 months of age in all 3 dogs.

Histopathology

The biopsy specimens taken at 5 months of age, revealed a subacute, severe interstitial nephritis in all 3 dogs. Lymphocytes were found in the interstitial tissue between tubules in both the cortex and medulla (Fig. 5). Some intratubular neutrophil accumulation was evident in Dog 80 while degeneration and necrosis of the proximal convoluted tubular epithelial cells were found in Dog 82 (Fig. 6).

It was evident from the needle biopsy that the nephritis was more severe and chronic in Dog 81 than in the other 2 dogs. Dense granulomatous inflammatory

Table 1: Creatinine, urea and PSP values

Dog	Age (months)	Creatinine µmol/ℓ	Urea mmol/ℓ	PSP (60 Min) µg/100 ml
80	4	129	12,3	No
	5	109	12,4	72
	6	128	8,9	90
	7	190	12,4	97
	8	153	9,3	90
	9	168	16,3	95
	10	177	13,3	98
	11	187	10,0	105
	12	198	13,2	No
81	4	194	24,6	No
	5	183	30,9	89
	6	170	14,0	102
	7	186	16,4	155
	8	177	15,2	110
	9	232	17,3	160
	10	218	27,8	140
	11	283	29,9	120
	12	375	33,1	No
82	4	136	13,4	No
	5	117	16,1	65
	6	147	9,4	91
	7	152	9,1	95
	8	183	7,8	56
	9	184	12,9	90
	10	157	12,1	94

No. not examined.
The normal creatinine values are below 133 µmol/ℓ while normal urea is 3,6 – 8,9 mmol/ℓ for the specific laboratory involved*
A PSP value lower than 80 µg/100 ml is normal for dogs[5].
*Section of Chemical Pathology and Haematology, Faculty of Veterinary Science, Department of Medicine, University of Pretoria, P O Box 12580, 0110 Onderstepoort.

infiltrates encroached upon Bowman's capsule while interstitial and periglomerular fibrosis were marked (Fig. 7). Glomerular sclerosis and atrophy were also prominent in the biopsy specimen from Dog 81.

At 7 months of age interstitial fibrosis and tubular atrophy, which are typical of chronic interstitial nephritis, were the dominant features found (Fig. 8). Glomeruli appeared atrophic and in some cases sclerotic while the spaces of Bowman were dilated. Severe medullary sclerosis could be demonstrated in all 3 dogs while hyaline casts were present in the medullary tubules of Dogs 80 and 81. Mineralization was demonstrated in the walls of some of the collecting ducts in Dog 81. Masson's trichrome stain confirmed the presence of mature connective tissue in the interstitial, subcapsular and periglomerular areas. No *Encephalitozoon* organisms could be demonstrated in any of the specimens at this chronic phases of the disease.

F. Radiopharmaceutical results

The right and left kidney parameters of time to peak (TP) and time of one half of the radioactivity to disappear from the kidneys (T½) as obtained for each of the 3 dogs and each study, are tabulated in Table 4.

Figs. 9 & 10 represent radiorenograms of two dogs (Dogs 80 and 81, respectively). The changes in kidney function which had occurred during the 3 months between the first (A) and second (B) studies can clearly be seen. Note that there were instances where the radiorenographic study did not run for the full time. This was due to movement of the dog which invalidated the remainder of the study.

Fig. 11 (A – D) depicts the DMSA scans of the kidneys of Dogs 80 and 81 taken for each with a 3

Table 2: Total serum proteins and serum protein electrophoreses levels

Dog	Age (months)	TSP g/ℓ	S-Alb g/ℓ	S-Glob g/ℓ	A/G ratio	S-Alpha-glob g/ℓ	S-Beta-glob g/ℓ	S-Gamma-glob g/ℓ
80	4	89,0	19,6	69,4	0,28	15,5	16,3	37,7
	5	76,0	26,3	49,8	0,53	16,1	17,5	16,1
	6	68,0	30,9	37,1	0,83	13,7	13,7	9,6
	7	60,0	36,1	43,9	0,82	16,7	16,7	10,6
	8	66,5	29,0	37,5	0,77	13,9	15,1	8,4
	9	65,0	30,5	34,5	0,88	14,2	13,2	7,1
	10	65,0	26,1	45,9	0,57	18,4	19,8	7,7
	11	70,0	25,7	44,3	0,58	17,5	19,8	7,0
	12	71,0	33,9	37,1	0,91	11,6	18,2	7,4
81	4	92,5	20,6	71,9	0,29	14,3	24,2	33,2
	5	72,5	21,8	50,7	0,43	14,9	20,9	14,9
	6	68,0	27,7	40,3	0,69	13,9	17,5	9,0
	7	64,5	27,8	36,7	0,76	13,9	15,2	7,6
	8	71,0	28,4	42,6	0,67	16,8	17,4	8,4
	9	72,0	31,1	40,9	0,76	16,9	16,9	7,1
	10	72,0	26,1	45,9	0,57	18,4	19,8	7,7
	11	70,0	25,7	44,3	0,58	17,5	19,8	7,0
	12	71,0	33,9	37,1	0,91	11,6	18,2	7,4
82	4	72,5	18,9	53,6	0,35	15,1	16,6	21,9
	5	66,0	24,0	42,0	0,57	16,5	14,9	10,8
	6	69,0	30,2	38,8	0,78	15,4	14,1	9,4
	7	68,0	29,9	38,1	0,78	14,6	15,2	8,3
	8	66,0	30,0	36,0	0,83	14,1	14,1	7,9
	9	65,0	30,5	34,5	0,88	13,2	13,2	8,1
	10	66,0	26,1	39,9	0,65	15,0	16,4	8,4
Controls	4	60,5	28,7	31,9	0,90	13,9	14,9	3,1
	7	64,7	31,2	33,5	0,93	13,4	13,6	4,5
	10	69,8	31,8	38,0	0,84	13,7	15,5	8,8
	12	73,3	34,4	38,8	0,89	14,9	17,9	6,7

Table 3: Urinalysis results: Dogs 80, 81 and 82

Date	20/2			6/3			17/3			10/4			5/5			15/6		
Dog number	80	81	82	80	81	82	80	81	82	80	81	82	80	81	82	80	81	82
S G	1,008	1,009	1,012	1,013	1,016	1,013	1,010	1,009	1,009	1,014	1,012	1,011	1,019	1,011	1,008	1,005	1,010	1,015
pH	6	6	6	6	6	8	7	7,5	6	7	6	5,5	7	7,5	7	6,5	6,5	5
Protein	1+	—	—	3+	2+	1+	1+	—	—	2+	—	—	1+	2+	1+	2+	1+	—
Glucose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Haemoglobin	—	—	2+	3+	—	2+	—	1+	—	3+	—	—	—	1+	—	—	—	—
Casts	1+	2+	1+	3+	3+	3+	3+	—	1+	1+	2+	—	1+	1+	—	2+	—	—
White blood cells	3+	1+	1+	1+	2+	2+	2+	2+	2+	3+	2+	1+	3+	3+	3+	1+	3+	1+
Red blood cells	—	—	2+	3+	1+	2+	—	1+	—	3+	1+	—	—	—	—	1+	1+	—
Tubular cells	—	3+	2+	1+	2+	2+	—	—	—	1+	—	—	—	—	—	—	—	—
Bladder cells	2+	2+	1+	1+	2+	1+	3+	1+	—	3+	—	1+	1+	2+	2+	1+	2+	1+
Bacteria	—	—	—	1+	1+	2+	3+	2+	1+	3+	3+	1+	3+	2+	2+	1+	3+	—
Encephalito-zoon spores	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

– negative 2+ moderate
1+ mild 3+ severe

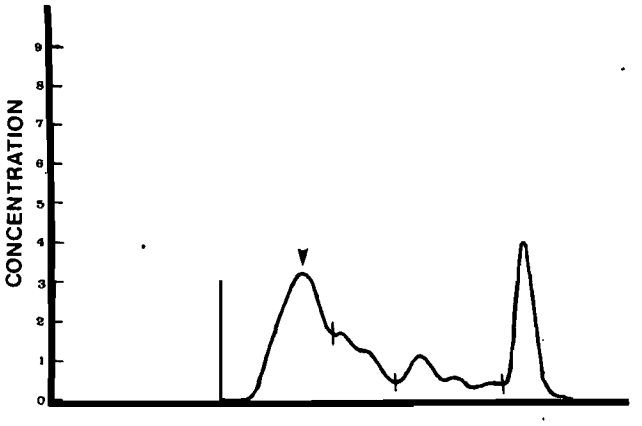


Fig. 2: Electrophoresis diagram at 2 months of age (Dog 80)

Table 4: TP and T½ values for DTPA-studies

Dog no		TP (min)		T½ (min)		Age (months)
		R	L	R	L	
80	A	2,16	—	19,02	—	5
	B	3,50	4,00	20,40	19,40	8
81	A	3,83	—	—	—	5
	B	5,33	8,33	—	—	8
82	A	3,0	3,33	13,44	14,03	5
	B	7,0	6,16	—	19,15	8

Legend: TP and T½ are presented here for left (L) and right (R) kidneys of each dog as obtained during the first (A) and second (B) DTPA studies. No value (-) indicates no measurable kidney function or excretion.

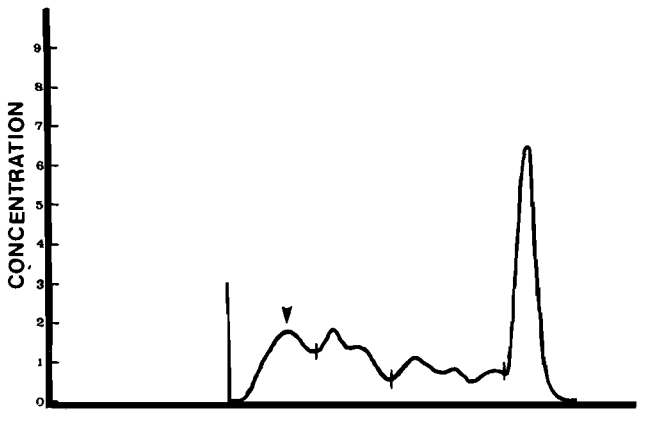


Fig. 3: Electrophoresis diagram at 3 months of age (Dog 80)

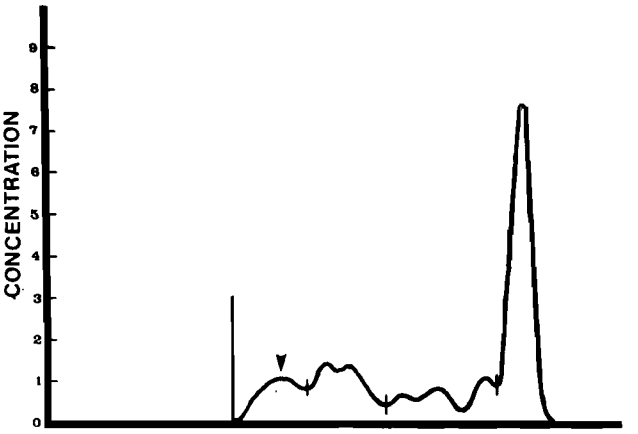


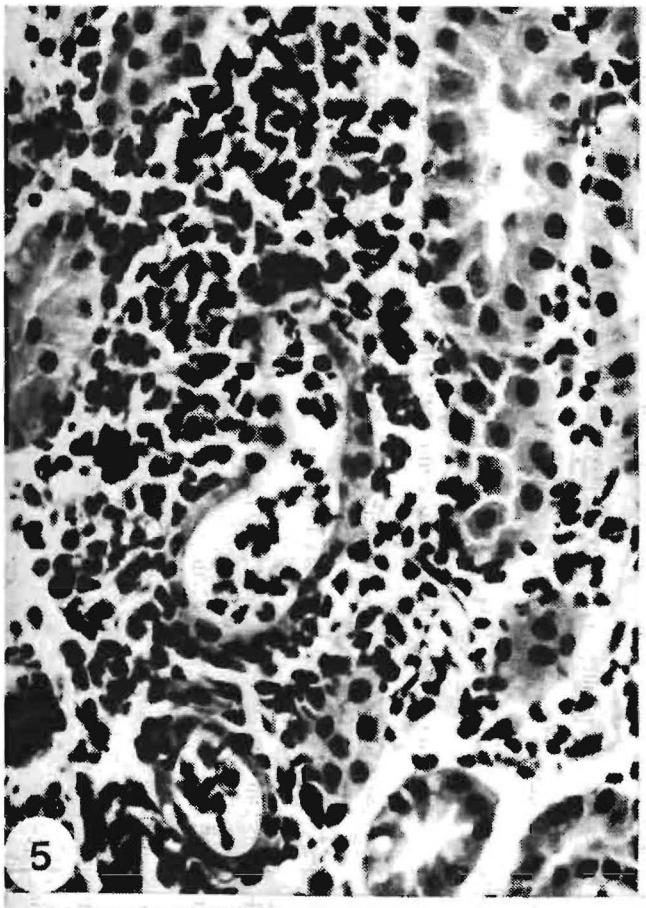
Fig. 4: Electrophoresis diagram at 9 months of age (Dog 80)

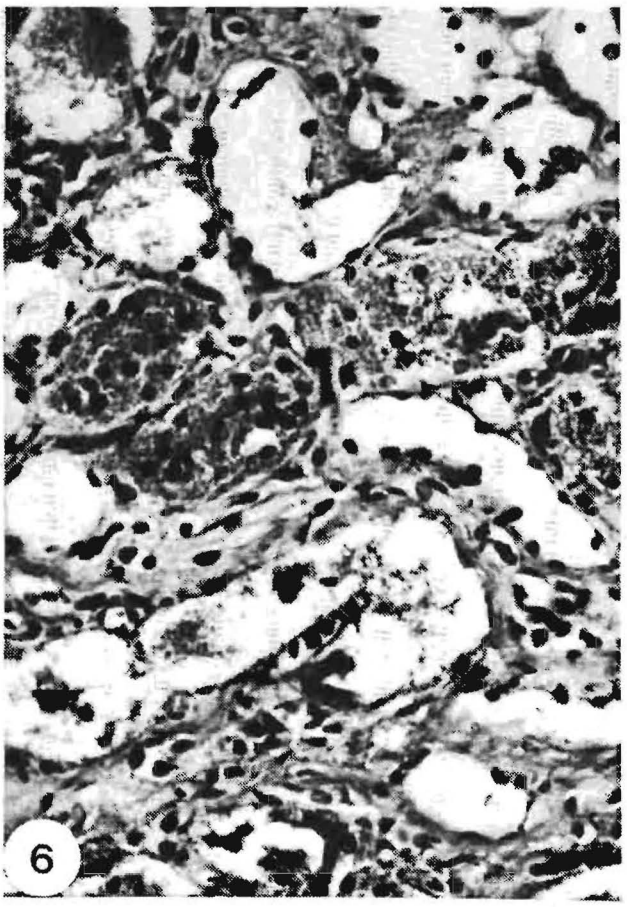
Fig. 5: The left kidney of Dog 80 shows numerous lymphocytes in the interstitial tissue while some neutrophils are found in the lumen of a renal tubule. HE X 600.

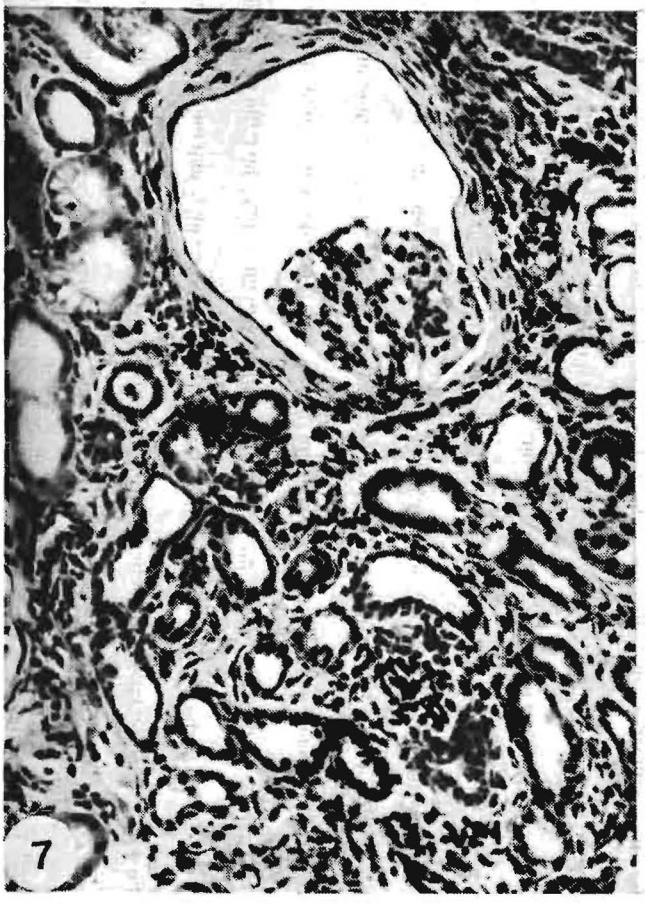
Fig. 6: Left kidney. Dog 82. Note the tubular epithelial cell degeneration and necrosis that is present. HE X 600

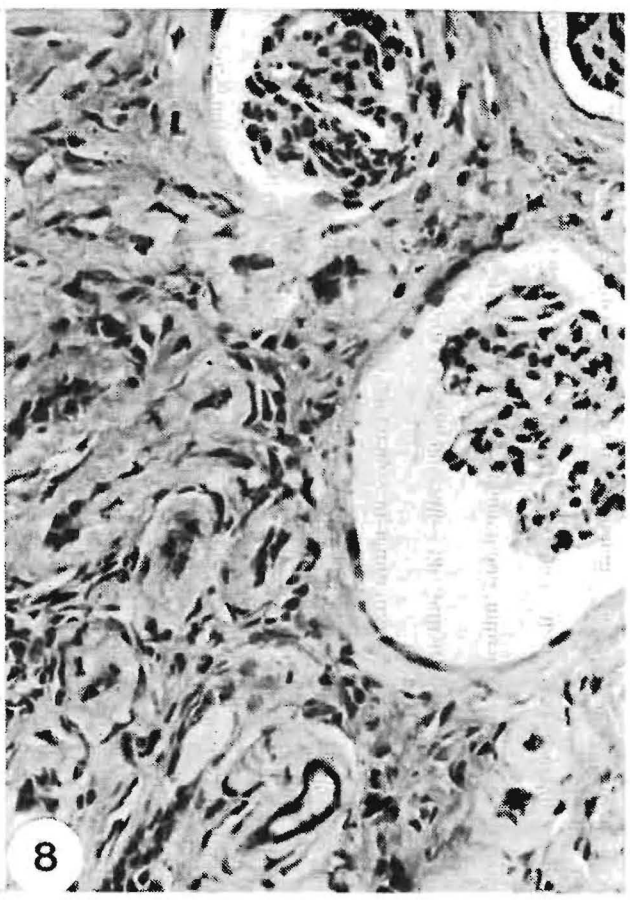
Fig. 7: Left kidney. Dog 81. Prominent atrophy of the renal tubules and glomeruli could be found. Periglomerular and interstitial fibrosis is also present as well as mild lymphocytic infiltration. HE X 200

Fig. 8: Right kidney. Dog 81. Severe periglomerular and interstitial cortical sclerosis could be found. HE X 400









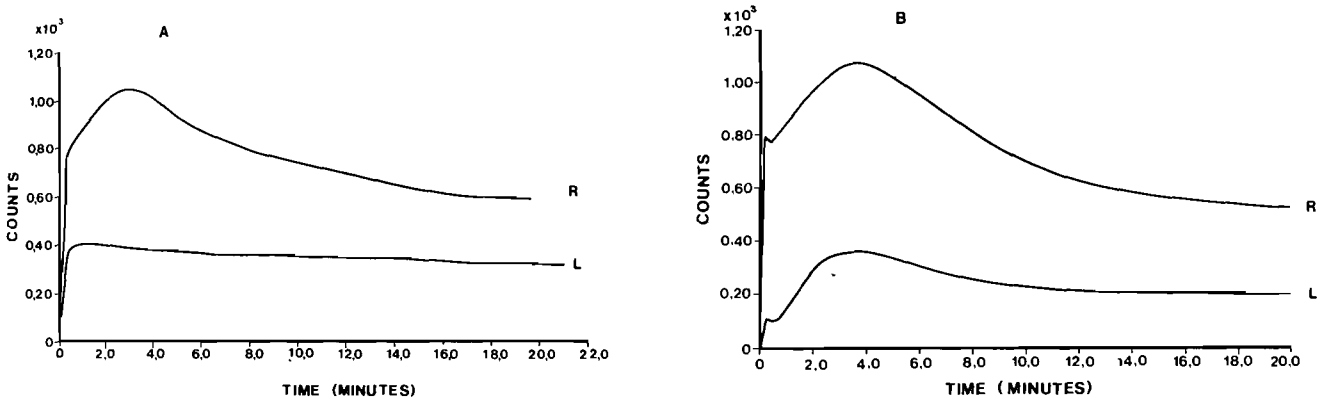


Fig 9 (A – B): Early and late DTPA renograms (left and right kidney) as obtained for Dog 80. The early left kidney curve (A) indicated no function.

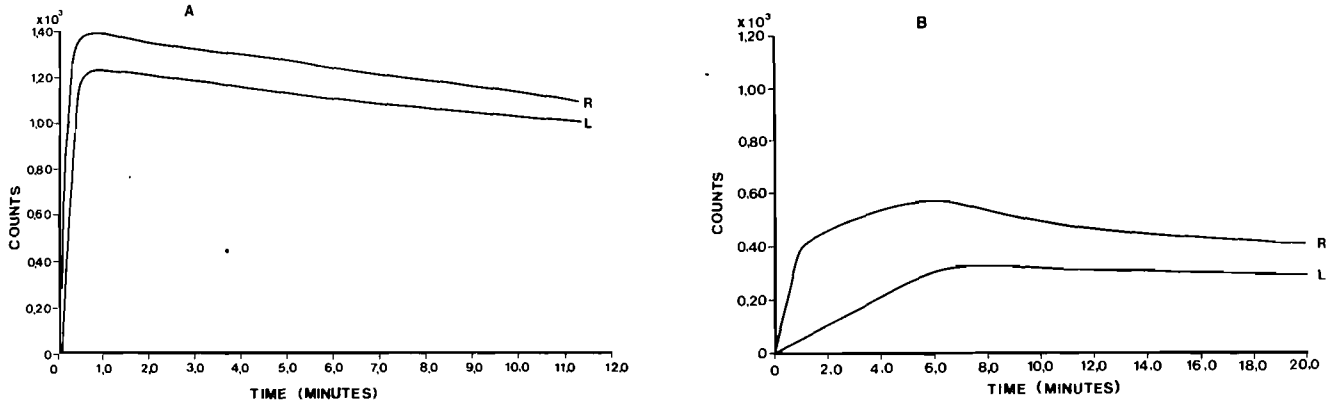


Fig 10 (A – B): Early and late DTPA renograms (left and right kidney) as obtained for Dog 81. Only the right kidney from the late scan (B) indicated some function.

month interval. Dogs 80 and 82 had a similar disease pattern based on the DMSA scans.

DISCUSSION

A poor growth rate was a prominent clinical observation in the three dogs suffering from the encephalitozoonosis. This may be a reflection of the chronic renal disease in these dogs. There was, however, very little other clinical evidence of disease in these dogs which emphasizes the fact that the disease can go unnoticed for a long time until renal failure finally sets in [1]. An initial drop in serum creatinine and urea levels were detected in all 3 animals which may be related to possible renal compensation. The eventual slow progressive increase in excretion products, however, indicates a final stage of inability of the glomeruli to clear the serum of these products.

The PSP retention test which specifically evaluates renal tubular epithelial damage, gave comparable results to the excretion tests[5].

The total serum protein (TSP) and electrophoresis (Table 2) showed a reduction in the TSP in the sequential tests in all 3 diseased dogs while the control animals had a mild increase in TSP. This reduction in TSP is directly related to reduced globulins with marked changes in the A/G ratio and specific reduction of the serum globulin fraction. The decrease in globulin is probably related to the recovery phase from active *Encephalitozoon* infection. *Encephalitozoon* spores disappear from the urine at about 3 months of age in neonatally infected dogs (W.S. Botha, 1983 Consultant Veterinary Pathologist, Pretoria, personal observation). An increase in serum albumin was also observed in the dogs suffering from encephalitozoonosis as well as in the control animals. The reduction in serum proteins does not appear to be directly related to the chronic renal disease.

Urinalysis proved to be essential for the detection of occult chronic renal disease. The urinary specific gravity (SG) was relatively low in all these cases indicating an inability of the kidney to adequately concentrate the urine. Isothenuria occurred at approximately the same SG as that of the glomerular filtrate (1.008 – 1.012) and was most marked in Dog 81. Mild persistent proteinuria, as seen in Dog 80, is also an indicator of urinary tract disease. The casts found in the urine sediment smears indicated severe kidney lesions. Excess tubular epithelial cells could also be demonstrated

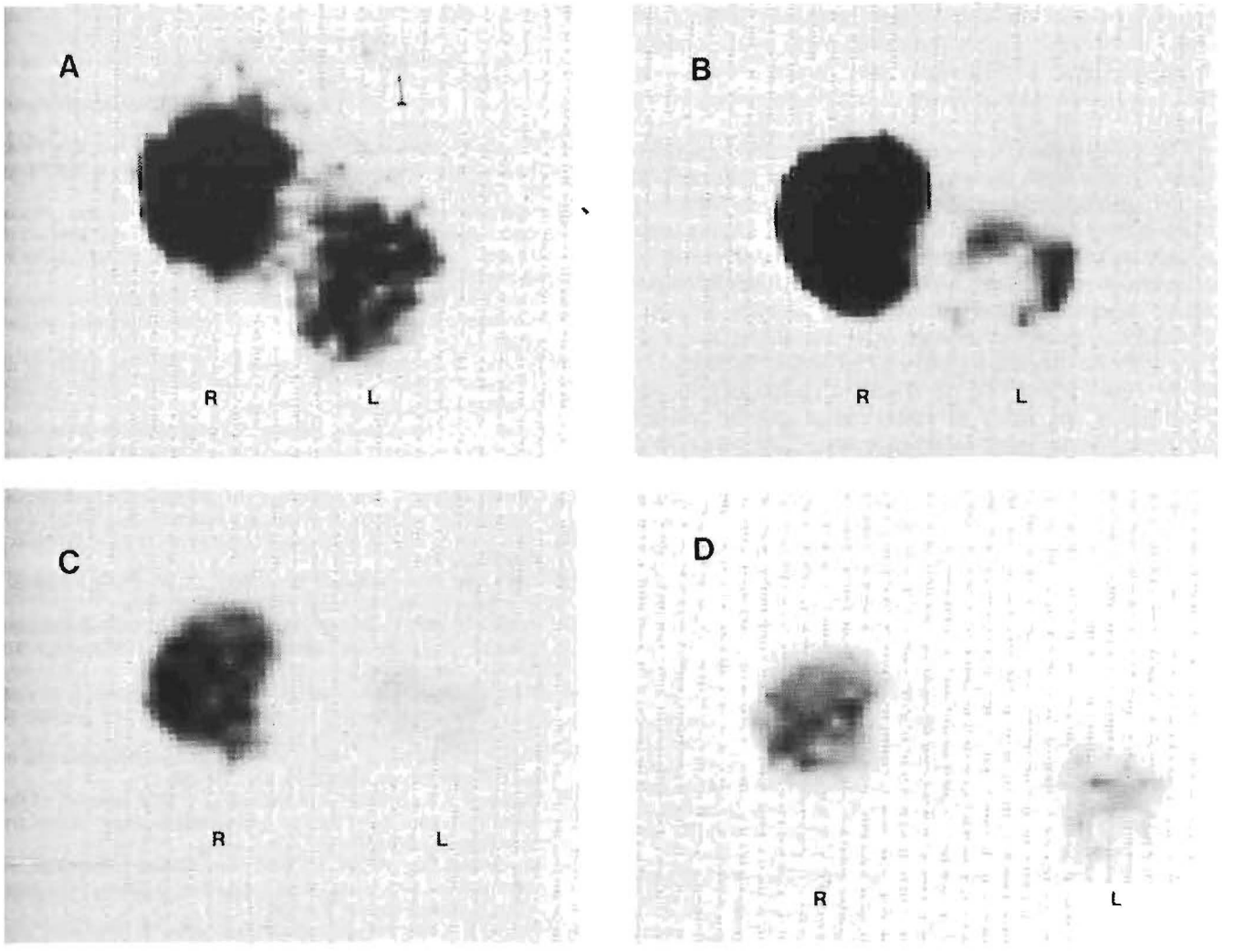


Fig. 11 (A – D): The DMSA scans represent morphological changes. Dog 80 (A and B) presented with marked deterioration of the left kidney, although function seemed to have improved. Dog 81 (A and B) indicated deterioration of both kidneys.

which further confirmed renal pathology. Casts were most numerous in the early examinations and disappeared from the urine in the chronic stages when collagen replaced normal tissue. The blood and bladder cells were not specific for kidney lesions and may occur in any urogenital tract infection. The bacteria found in the urine were most probably related to concomitant infection and contamination of specimens. The presence of relative large numbers of white blood cells in most of the urine specimens are probably related to a concomitant bacterial cystitis.

Kidney biopsies rendered valuable information while the laparotomy and visual examination of the kidney surfaces gave information on the severity of the condition. It was possible, on histophathology, to visualise glomerular, tubular and interstitial morphological changes and to get an indication of the chronicity and possible irreversibility of the renal pathology. At 7 months of age the amount of mature collagen (positively stained by Masson's trichrome stain) indicated irreversible and further progression of chronic disease in all 3 dogs. The severity of the morphological changes were in correspondence with the disturbance of kidney function as indicated by the kidney function tests and urinalysis.

Renographically Dog 80 had relatively poor kidney function at 5 months of age. In the right kidney the function was poor as was indicated by the clearly defined TP and evinced by a definite although prolonged T½. By the same standards, the left kidney was virtually non-functional. Three months later the left kidney from Dog 80 had recovered some of its function as indicated by a measurable TP value, while the status of the right kidney was unchanged. The kidney function in Dog 81 at 5 months of age was poor in both the left and right kidneys (Fig 10A) and remained unchanged for the next 3 months. The initial DTPA study at 5 months of age in Dog 82 indicated reasonable kidney function for both kidneys, but slow excretion occurred (Table 6). At 8 months, both kidneys of Dog 82 revealed a deterioration in excretory function.

Based on the DMSA-scans, the right kidney of Dog 80 and Dog 82 seemed to be reasonably unaffected at 5 months of age. The ragged left kidney pointed to sclerosis of the medulla and the cortex. After 3 months the right kidney seemed unchanged but there was a marked deterioration in the left kidney. The first DMSA-scan from Dog 81 showed that both kidneys had been affected; the right kidney less so (Fig 1C). After 3 months the right kidney appeared to have deteriorated

markedly while the left kidney remained unchanged (Fig. 11D). The morphological changes evident from these sequential DMSA-scans gave further evidence of the progressive renal sclerosis in canine encephalitozoonosis.

The differentiation of potentially reversible, primary renal disease from progressive, irreversible failure is of great significance in the treatment of dogs suffering from kidney failure. Some of the available kidney tests appear to be insensitive and the diagnosis of irreversible progressive renal failure is therefore limited to the stages where advanced renal pathology is already present. Careful interpretation of sequential creatinine, urea and PSP retention values, urinalysis and kidney biopsies are at the moment some of the standard measures that the practitioner can apply to assess the prognosis in dogs suffering from encephalitozoonosis. The additional employment of radiopharmaceutical techniques will give further indication of renal dysfunction. The radioisotope studies have the advantages of firstly being a non-invasive follow up on the progression of the disease and secondly of being able to differentiate between the function of the left and right kidneys.

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ARTICLE ARTIKEL

PATHOGENESIS OF SUBCLINICAL BOVINE MASTITIS: DIAGNOSTIC AND DYNAMIC CHARACTERISTICS OF VARIOUS SUBCLINICAL UDDER CONDITIONS MONITORED BY MEANS OF THE INTERNATIONAL DAIRY FEDERATION CRITERIA

W.H. GIESECKE, MARIE-LUISE BARNARD and MAGDALENA G. MENDELOVISH**

ABSTRACT: Giesecke W.H.; Barnard M-L.; Mendelovish M.G.. **Pathogenesis of subclinical bovine mastitis: Diagnostic and dynamic characteristics of various subclinical udder conditions monitored by means of the International Dairy Federation criteria.** - *Journal of the South African Veterinary Association* (1986) 57 No. 2, 87-90 (En) Veterinary Research Institute, 0110 P.O. Onderstepoort, Republic of South Africa.

This investigation, conducted on a small herd milked by machine without disinfectant teat dipping, shows that a limited population of 21 normally lactating dairy cows regularly examined at 24 h intervals during 22 consecutive days, may be used for monitoring dynamic fluctuations of udder conditions. From the results it is apparent that, on subclinical levels, udder health of dairy cows depends in principle on a variety of conditions, variability in dynamic fluctuations and the balance between persistent, deteriorating and improving health states. As health states, especially active and intermediate to the mastitic and normal condition at the opposing extremes of udder health, aseptic mastitis (AM) and latent infection (LI) cross-reacting with each other and with each of the other health states, are of particular practical interest. From the dynamic fluctuations of AM and LI it seems conceivable that the strategy of mastitis control proposed by several workers and found lacking in several respects during this investigation, may promote the shifting of abnormal udder conditions from those associated with mastitogenic infections to others of a non-bacterial, i.e. unspecific nature. In general, the data suggest that further work on the dynamics of different health states is necessary, and that it may be advantageous also to use the small herd model described for determinations on representative populations of cows selected at random from large dairy herds otherwise unsuitable for investigations on daily and other dynamic changes of udder health.

Key words: Subclinical bovine mastitis, International Dairy Federation criteria, diagnosis, pathogenesis.

INTRODUCTION

Various workers[1 3 9] have in the past referred to the dynamics of udder infection on the herd level, emphasizing the importance thereof in the definition of mastitis[9], its role in the formulation of a strategy of mastitis control[1 2 9] and the general scarcity of data available[3]. The core of the proposed strategy for mastitis control has been expressed by means of the equation A = B x C x1/100, where A = average numbers of cows (as % of the total number of cows) infected at a given time; B = D + E, where B = numbers of cows (as % of the total number of cows) infected at any time during a specific period; D = numbers of cows (as % of the total number of cows) infected at the start of the period and E = numbers of cows (as % of the total number of cows) uninfected at the commencement, but becoming infected during the period, and C = mean duration (as % of specific period) of infection during the period[1 2].

The equation indicates that the strategy proposed in principle revolves around the assumption that mastitis associated with udder infection, is the only type of mastitis, clinical and subclinical. The strategy of mastitis control is further based on investigations into the dynamic fluctuations of subclinical udder infections in a large population of dairy cows apparently monitored at the commencement and the conclusion of a 12-month period[1]. Because of the latter the results from 721 cows, 26% of which were free from infection and 45% of which were infected at the start and the end of the year, have been interpreted as indicating health conditions of extended stability[1].

**Veterinary Research Institute, 0110 P.O. Onderstepoort, Republic of South Africa.

However, stability during the prolonged period of investigation may have been accidental as it is conceivable that the cows, during the course of the investigation, may have been subjected to repeated but un-observed changes in their udder health. Because of this possibility, and the rather limited data available on the dynamics of subclinical udder infection in dairy herds[3], an investigation into the dynamic fluctuations of subclinical conditions during short periods of 24 h each, was indicated.

MATERIALS AND METHODS

This investigation was designed as a model for a small herd milked by machine without disinfectant teat dipping, where during their normal lactation period the same 21 cows with 83 functional quarters were examined on 22 consecutive days, subdivided into 21 separate udder health monitoring herd examinations (UHMHE-s) which were conducted during periods of 24 h each as already described elsewhere[6] using the recommended cytological and bacteriological parameters and criteria[8].

RESULTS

General diagnostic and supplementary characteristics of udder conditions on the subclinical level

The investigation showed 4 udder conditions which differed, respectively, in their general prevalence, diagnostic and supplementary characteristics (Table 1).

The udder condition (Table 1) persisted for only a short time in quarters affected with mastitis (M), latent infection (LI) and aseptic mastitis (AM), whereas in the unaffected, apparently normal quarters it persisted for a surprisingly long period since bacterial challenge was

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Table 1: Prevalence, diagnostic and supplementary characteristics of 4 udder conditions determined by means of the IDF* criteria on the 1826 udder quarters investigated

Designations	Udder health states x values			
	M**	AM	LI	N
General prevalence of conditions (numbers of quarters involved)	441	94	319	972
Pathogenic bacteria in aseptically collected foremilk quarter samples	Present	Absent	Present	Absent
$\bar{x} \pm$ SD+ values of SCC*x10^3/ml of milk	2610 ±2570	3955 ±4110	168 ±136	82 ±83
$\bar{x} \pm$ SD values of diameters (mm) of BSA* precipitation zones	7.12 ±1.74	6.48 ±1.01	6.14 ±0.92	5.99 ±0.92
$\bar{x}$-persistence (days) of bacteriologically and otherwise diagnostically identical states of udder health++	2.68	1.44	2.69	5.96

*International Dairy Federation
**M = mastitis; LI = latent infection; AM = aseptic mastits N = normal; SCC = somatic cell count; BSA = bovine serum albumin
+Mean ± standard deviation
++NB. Changing from one to another health state does not necessarily mean that an affected quarter becomes normal and *vice versa*, nor does it preclude recurrence of the initial condition

presumably promoted through milking by machine and the omission of disinfectant teat dipping immediately after milking. However, irrespective of such differences, the results on persistence of the condition generally indicate that at the subclinical level all health states may be more or less transient. The transiency of health states does not become apparent from comparing the respective prevalence of such states at different investigations, but by comparing quarter by quarter, all the changes of conditions monitored. As shown below (Table 2) the point prevalences as such of conditions at the start and the conclusion of the 24 h intervals of this investigation, only indicated a quantitative change of condition in 0,92% of quarters while during the 24 h intervals qualitative changes of conditions occurred in 18,93% of the 1743 quarters monitored.

Dynamic fluctuations of udder conditions during periods of 24 h each

Redistribution of quarters within their respective groups related to a particular health state (Table 2), suggests that udder conditions determined by means of the IDF criteria, were subject to marked fluctuations during the 24 h intervals between corresponding herd examinations.

In general, the results indicate a balancing of udder conditions with initially determined health states persisting in 1413 quarters, improvement in 162 and deterioration in 168 other quarters. The initial and final point prevalence of each of the 4 conditions therefore only showed very limited quantitative changes despite the fact that during the 24 h periods considerable fluctuations occurred in 330 of the quarters (Table 3).

Table 2: Distribution of conditions determined by means of the IDF criteria on 1743** udder quarters monitored

Udder conditions (UC) and distribution of quarters		Udder conditions and redistribution of quarters during 24 h intervals between the initial and final investigations of 21 consecutive UHMHE-s			
At start of 24 h intervals					
UC	Numbers of quarters	M	AM	LI	N
M	425	338*	16	58	13
AM	87	19	25*	10	33
LI	301	58	6	199*	38
N	930	9	33	37	851*
At conclusion 24 h intervals: Numbers of quarters		424	80	304	935

*Quarters with udder conditions persisting unchanged during a 24 h period. N.B.: Quarters redistributing above and below diagonal line of * indicate improving and deteriorating conditions
**For explanation of difference between 1826 quarters investigated and 1743 quarters monitored, see[6]

Table 3: Initial distribution and corresponding redistributions of conditions determined by means of the IDF criteria on the 1743 udder quarters monitored*

Udder conditions (UC) and distribution of quarters		Relative redistribution of quarters during 24 h intervals between the initial and final investigations of the 21 consecutive UHMHE-s calculated where IPP values = 100% each of corresponding udder conditions			
At start of 24 h intervals (= initial point prevalence = IPP):		%IPP			
UC	Numbers of quarters	M	AM	LI	N
M	425 (= 100%)	79.53*	3.77	13.65	3.06
AM	87 (= 100%)	21.84	28.74*	11.49	37.93
LI	301 (= 100%)	19.27	1.99	66.11*	12.63
N	930 (= 100%)	0.97	3.55	3.98	91.51*
At conclusion of 24 h intervals: Numbers of quarters		(424)	(80)	(340)	(935)

*For further detail see Table 2

From the results (Table 3) it is clear that out of the 425 quarters initially mastitic, 79,35% remained mastitic, 3,76% developed AM and 13.65% LI, whereas 3.06% became normal. Similar fluctuations of udder health and redistributions of quarters were also monitored in the groups of quarters initially showing AM, LI and N.

The incidences of new cases of AM and LI (Table 3) suggest that these conditions may develop in either improving or deteriorating udder health. The groups of quarters with persistant and newly developed AM and LI, thus represented the dynamically most active pools of quarters with health states intermediate to the 2 extremes of udder health, namely the mastitic and normal conditions (Fig. 1).

Judged by its limited persistence in only 28,74% of in-

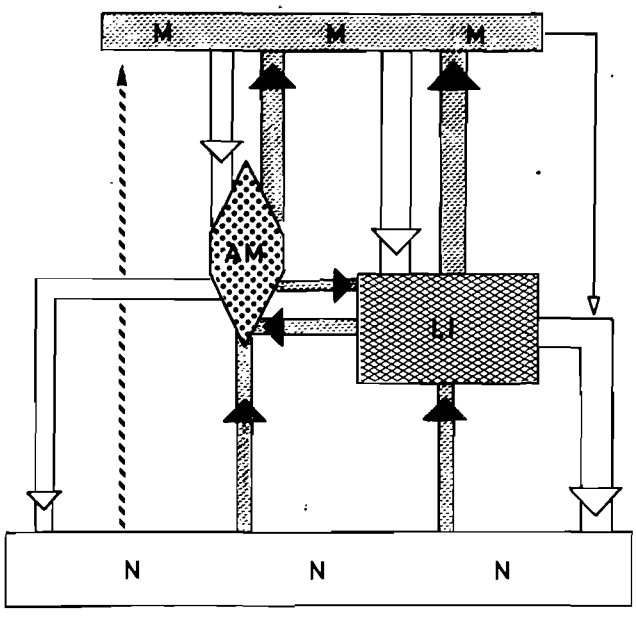

Fig. 1: Schematic presentation of the dynamic balance during 24 h periods of 4 inter-dependent subclinical health states daignosed by means of the double-parameter IDF criteria and represented by the corresponding pools of quarters with M (shaded area), AM (dotted area), LI (meshed area) and N (unshaded area), each subject to fluctuations of persistent (areas), improving (unshaded arrows) and deteriorating (shaded arrows) udder conditions. The arrows indicate the dynamic changes of conditions along certain pathways apparently related to the deterioration and restoration of bovine udder health as suggested by results in Table 2 above.

itially affected quarters (Table 3), AM may be considered to be a particularly labile condition. The pool of quarters with AM received new cases from pools of quarters with the initial conditions N, M and LI (Table 3), whereas it, in turn, emitted in similar order new cases to the same pools of quarters (Table 3).

In contrast, the pool of quarters with persisting LI seemed more stable. It received new cases from pools of quarters initially showing M, N and AM (Table 3) and it, in turn, emitted in similar order new cases to the same pools of quarters (Table 3).

DISCUSSION

From the 4 udder conditions which have been distinguished during this investigation by the IDF criteria[8] and which differed from one another in their general prevalence, mean persistence (Table 1) and dynamic fluctuations (Table 2 & 3) during the 24 h intervals between corresponding herd tests, it is evident that, on the subclinical level (Fig. 1), udder health depends upon a variety of conditions, variability in dynamic fluctuations and the dynamic balance between persistent, deteriorating and improving health states.

It is therefore clear that during normal lactation and depending on other conditions, bovine udder health may be regarded as extremely labile, subject to frequent fluctuations and highly sensitive to bacterial challenge like that facilitated during this investigation. An extended stability of udder health conditions as suggested[1] therefore seems more the exception than the rule and is probably more closely related to artificial than to natural circumstances. Further, the equation-like synopsis of the strategy of mastitis control generally proposed[1-3] seems rather over-simplified, for reasons, such as: (i) subclinical udder infection, a condition presumably considered synonymous with subclinical mastitis, has not been defined appropriately and the terminology is therefore as confusing as that of intramammary infection; (ii) the corresponding research findings referred to [1-3] have not been supported by data conclusively verifying the truly intramammary nature of the udder infections reported; (iii) the variable E of the equation is applicable to new cases of udder infections, but the equation as such neither makes provision for the deterioration nor the improvement of udder health in the absence of udder infections.

It is clear from this investigation that AM and LI are important conditions cross-reacting with each other in an intermediate position between the 2 extreme poles of udder health on the subclinical level, namely, mastitis on the one end of the scale and normality on the other. It is further evident that major sources of AM were the pools of quarters with N, M and LI (Table 2) which supplied 41,25%, 20,0% and 7,59% respectively, of new cases of AM. This suggests that a strategy of mastitis control which is similar to the one proposed[1-3] and is aimed exclusively at the elimination and prevention of so-called udder infections, may promote the shifting of udder health problems from the usually bacterial to the non-bacterial abnormal health states like AM (also termed unspecific mastitis[8] or secretory disturbance[10]). In fact, such shifting has already been reported[7], and it is also apparent from investigations conducted on thousands of quarters[10]. The latter results on changes in udder conditions during 1973/1980, show that on average, quarters with M, AM (diagnosed as non-bacterial secretory disturbances) and LI decreased by 49,09%, 11,90% and 29,24%, respectively, whereas normal (N) quarters increased by 12,49%. Quarters with AM and LI clearly responded to the control measures different to the quarters with M. Although it is conceivable that some portion of the cases of AM might have been associated with anaerobic bacteria which normally cannot be readily determined[4,5], it seems improbable that such micro-organisms were the major cause for the disproportionately limited reduction of AM mentioned above[10].

Apart from their significance as health states intermediate to subclinical mastitis and normality, AM and LI further seem particularly interesting because the respective pools of quarters affected (Table 2 & 3) were each a source of almost equal importance for 2 opposing developments, namely, that of new cases of M and that of newly normal quarters (Fig. 1). Neither the quarters with AM nor those with LI showed dynamic fluctuations which suggest a particular association with one or the other of the extreme poles of udder health at the subclinical level. Although the pool of quarters with AM seemed to emit more new cases of LI (Table 2) than the reverse, both pools of AM and LI seemed related to the same degree to dynamic fluctuations at the normal and at the mastitic pole (Table 3). This non-polarization of health changes associated with the pools of quarters with AM and LI seems as confusing as the atypical mean values of lacteal levels of BSA (Table 1) related to the 4 health states distinguishable by means of the IDF criteria. However, the clarification of such a confusion is feasible from further determinations by means of the IDF/BSA criteria used in parallel to the IDF criteria on the same milk samples.

ACKNOWLEDGEMENTS

Advice and assistance of Dr H van Ark (Department of Agriculture and Water Supply, Division of Datametric Services) on statistical aspects of the investigation are gratefully acknowledged.

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ARTICLE ARTIKEL

A FIELD TRIAL COMPARING THE EFFICACY OF SULPHAMONOMETHOXINE, PENICILLIN, AND TARANTULA POISON IN THE TREATMENT OF *PODODERMATITIS CIRCUMSPECTA* OF CATTLE

S. STAMPA*

ABSTRACT: Stampa S. **A field trial comparing the efficacy of sulphamonomethoxine, penicillin, and tarantula poison in the treatment of *pododermatitis circumspecta* of cattle.** *Journal of the South African Veterinary Association* (1986) **57** No. 2, 91-93 (En) Department of Animal Science, University of Fort Hare, Private Bag X1314, 5700 Alice, Republic of Ciskei.

Nearly 80% of 87 cattle suffering for the first time from *pododermatitis circumspecta* were cured by each of the three drugs under test. Sixty and 73% of those cured by sulphamonomethoxine and penicillin, respectively, and 29% of those cured by the tarantula poison (Theranekron**), showed relapses within 6 months. Of the 92 cattle with previous records of *pododermatitis circumspecta*, sulphamonomethoxine cured 44%, penicillin 73% and Theranekron 32%. Of the latter three groups 72 – 80% showed relapses within the subsequent 6 months. The results of surgical treatments were, possibly, improved by the prior administration of Theranekron. In addition, in a pilot trial, the demarcation of a gangreneous udder half of a goat suffering from blue-bag, appeared to be accelerated by the parenteral administration of Theranekron.

Key words: Pododermatitis circumspecta, treatment, cattle, tarantula poison.

INTRODUCTION

Pododermatitis circumspecta of cattle, as defined by Greenough et al.[6], occurs frequently in South African dairy cows.

Blunt trauma to the hoof corium on account of heavy weights, abnormal posture or excessive length of the hoof, specific feeds, unsuitable stable floors, thrombosis of the supplying arteries, preceding laminitis and inherited disposition have been suggested as possible causes[6].

The lateral hind toe is most frequently, but not exclusively, affected. The disease begins as haemorrhage at the sole or the sole-heel junction of the corium. Bacteria commonly invade the damaged tissues, leading to inflammatory reactions. Granulation tissue is formed. It may protrude through the horn of the sole, thus forming the lesion known as the *"Rusterholz'sches Sohlengeschwür"*. The infection readily spreads to the pododerma of the surrounding tissues, leading to a septic laminitis with extensive underrunning of the sole. In complicated cases, it may reach deeper tissues, such as the navicular bursa, the deep flexor tendon, the pedal joint or even the deep flexor tendon sheath[6].

The disease is very painful causing severe lameness. Milk production and condition of affected animals drop. Bilaterally affected cases may show a tender pace but no striking lameness. In these, the condition may remain hidden for several days, only to be noticed when signs of general illness or swelling of the corona become evident.

The diagnosis is obvious in all cases in which the granulation tissue has broken through the sole. Early cases of the disease are diffucult to distinguish from other inflammatory conditions of the pododerma. Red- or grey-stained spots, *"Steingallen"*[10], are usually exposed during paring of the sole in *pododermatitis circumspecta*. Such spots are absent in other inflammations of the pododerma and may be accepted as diagnostic for this disease.

A very rare case may heal spontaneously. The disease usually persists for months or years, unless treated.

Surgical treatment has been the standard approach for years and must be resorted to in all serious cases[4 6 9 10]. All the necrotic horn and all the apparently healthy horn which is not firmly attached to the pododerma must be removed. The inflamed and necrotic tissue as well as the granulation tissues are resected. Fistulous canals leading to pus-filled cavities are opened wide. A smooth surface of healthy tissue is created, irrespective of whether the lesions were limited to the pododerma of affected deeper structures as well. After the operation, the edge of the soft-tissues must be continuous with healthy horn. The wound has to be dressed and bandaged. The bandage must be changed after 48 hours to check for diseased tissue or pus which have remained undetected during the operation. The treatment is in most cases rewarding. It involves a great deal of work and is cumbersome on account of the resistance offered by patients in spite of local or general anaesthesia.

Alternative approaches have been tried. The control of bacterial infection in the tissues damaged by haemorrhage by means of systemically applied sulphonamides or penicillin or other anti-microbials, can be rewarding[3 6 7 9 10]. If applied early whilst neither surface lesions nor significant swellings around the corona have developed, it may lead to a complete cure. In less successful cases, it may cure the infectious pododermatitis in the vicinity of the main lesion and improve lameness. However, these patients' feet are bound to remain tender, and once anti-microbial treatment is discontinued, the pododermatitis in the adjoining tissues is likely to flare-up again.

A further alternative is the employment of Theranekron (R). This drug is, according to the producers, a sterile injectable solution containing 10 mg tarantula poison per ml. It is prepared from the spider *Tarantula cubensis*. Details of the production process have not been released.

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**Theranekron is the registered trade name of Messrs Therapogen Werk, Tölzerstr. 1, 8022, Grünwald, German Federal Republic.

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This spider poison is said to stimulate the demarcation prosess by which, in the course of inflammation or necrosis, dead or infected tissue is separated from the healthy tissue[7,8]. Several authors[3,4,7,9] have reported encouraging results with this drug in hoof inflammations. It is claimed to offer an additional advantage in that no drug residues are found in milk and meat, so that these products can be used without restrictions[7]. This advantage, if confirmed for South African conditions, could become significant in this country as well.

MATERIALS AND METHODS

Pilot trial

A goat ewe suffering from *mastitis gravis* (blue-bag) in one udder half became available for studying the promotion of the demarcation process. The affected udder section was as hard as rock and its skin discoloured blackish-blue. Neither milk nor inflammatory products could be milked from its teat. The patient had no appetite. The rectal temperature was 40,7°C. According to own experience, such animals usually die, unless mastectomised. Beer[1] states that up to 90% die and that survivors demarcate the affected parts within 5 – 8 weeks.

The patient was treated with one injection each of 5 ml ampicillin (0,15 g/ml) and 2 ml Theranekron. The appetite returned within 24 hours. Five days after the treatment, a red demarcation line became visible. It surrounded the affected quarter at its base and along the median grove. After a further five days, separation had proceded so far that light traction caused the diseased tissue to fall off. Only two portions of apparently healthy udder tissue remained attached to the abdominal wall. The one was 20 x 120 and the other one 20 x 40 mm large. The separated gangrenous udder tissue showed two holes at the corresponding sites. Epithelium covered the demarcation surface in due course with the exception of the larger of the two remaining stumps. When this was resected, the wound closed.

Main trail

All dairy cows of the herd of the University of Fort Hare which were reported to be lame, were used in these tests. The work was performed between 1981 and 1984 and consisted of 179 individual treatments. All animals were affected in the hind legs. The lame leg was lifted, the hoof cleaned and inspected. Hooves with a flat sole, the original concavity having been lost, were prepared superficially. If grey or red spots became visible, the animal was assigned to the trial.

One of the following treatment schedules was used:

1. Fifty ml of a 20% injectable solution of sulphamonomethoxine (Daimeton, Daiichi Seiyaku)
2. Twenty five ml of a mixture containing 150 000 i.u. (= 142 mg) benethamine penicillin and 150 000 i.u. procaine penicillin per ml (Compropen, Glaxo)
3. Ten ml of an injectable solution containing 10 mg tarantula poison per ml (Theranekron; Therapogen-Werk)
4. Twenty two cows were kept as untreated controls.

The animals were, generally, assigned at random to these treatments and a control left untreated, when it was acceptable to the dairy manager. Shortage of drugs, however, necessitated deviation from randomisation at times. Thus, more patients were subjected to the penicillin treatment than to the other two drugs. For details refer to the Table 1. All animals were seen twice a day for the complete experimental period. The clinical success was juged by observing the patient walking. Tree categories were distinguished: cured, well improved and not improved.

Animals cured, i.e. walking without signs of lameness at any stage after treatment, usually within 3 – 7 days, were used for further tests, if becoming lame again at a later stage. Animals which had improved but were not cured, were discarded from further tests. Untreated controls and animals which had failed to improve after the treatment were either subjected to surgical treatment or sold for slaughter.

For the surgical treatment, patients were anaesthetis-

Table 1. Results of the treatments

Treatment	Number of cattle													Relapse observations					
	total in test	treated once		treated twice		treated more often		cured		improved		not improved		observation time after treatment too short available for observation of relapse		ill again		not ill again	
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	n	n	%	n	%
A Cattle ill for the first time																			
Daimeton	9	9	100	0	0	0	0	7	78	1	11	1	11	4	5	3	60	2	40
Compropen	64	46	72	5	8	13	20	49	77	9	14	6	9	9	55	40	73	15	27
Theranekron	14	12	86	1	7	1	7	11	79	1	7	2	14	7	7	2	29	5	71
Controls	22	—	—	—	—	—	—	1	5	0	0	21	95	—	—	—	—	—	—
B Cattle with records of previous hoof diseases																			
Daimeton	16	9	56	5	31	2	13	7	44	1	6	8	50	11	5	4	80	1	20
Compropen	48	39	81	8	17	1	2	35	73	8	17	5	10	9	39	28	72	11	28
Theranekron	28	19	68	2	7	7	25	9	32	9	32	10	36	13	15	12	80	3	20
C Cattle treated surgically after drug treatment																			
Daimeton	6							2	33			4	67						
Compropen	3							1	33			2	67						
Theranekron	9							5	55			4	45						

ed by the intravenous administration of 2 ml xylazine (2mg/ml) (Rompun, Bayer) plus 2 ml propionylpromazine (2,88 mg/ml) (Combelen, Bayer). The patient was cast and a nerve block of 5% procaine with adrenalin applied, as recommended by Berge & Westhues[2]. Haemostasis was produced by tightening a rubber tube over the metatarsal region. The lesion was excised and bandaged as described above. In three patients in which the inflammation had spread to the deep flexor tendon sheath, the affected toe was amputated according to the method recommended by Geiger (personal communication). The amputated stump is in this technique not covered by a skin flap, as recommended by Berge & Westhues[2] but left open to heal under a pressure bandage *per secundum*. Under field conditions, the author found Geiger's procedure more successful than the standard method.

RESULTS

The results of the trail are presented in Table 1.

Nearly 80% of all cows ill for the first time were cured by each of the three drugs tested. Fewer Theranekron-treated animals of this group became ill again than those treated with the sulphonamide or penicillin. This observation may indicate that Theranekron eliminated the pathogens or affected tissue better than the antimicrobials.

A smaller percentage of patients with records of previous hoof inflammations were cured by sulphamonomethoxine and the tarantula poison than animals ill for the first time. Penicillin was superior in these patients relative to the former two. However, after apparent cures, many of these repeater patients suffered a relapse. In this respect, all three drugs showed similar results. It must be assumed that either the cause had not been corrected or the affected tissue not been eliminated by the treatment.

One of the 22 controls cleared up clinically, the other 21 animals failed to improve without treatment.

Whether the surgical treatment was favoured by the previous treatment with Theranekron, as indicated in Table 1, remains uncertain.

DISCUSSION

The technique employed in the main investigation has some limitations. It could have been improved by excluding patients with advanced lesions which had been lame for several days. Such cases occurred with bilateral involvement and, occasionally, after weekends. They could possibly have reacted differently to drug treatment than early cases and this might have influenced the results. Furthermore, had the same number of animals been subjected to each drug, evaluations of results would have been easier. Also a method of assessing the improvements accurately, instead of the subjective judgement used, could have rendered the results more precise. However, some useful information has been obtained in spite of these shortfalls.

The results reported in this study resemble those found by earlier investigators[7 9] though being less successful than those reported by other[3 11]. They may be more reliable than those of previous investigators, in that a greater number of animals was subjected to treatments, three different drugs were compared with each other, some controls were kept, patients with previous records of hoof inflammations were dealt with separatly from new cases and a long observation period permitted detailed observations on relapses.

The rather complicated structured diseased udder tissue of the goat demarcated more rapidly than expected. This observation supports the claim that Theranekron speeds up demarcation[1 7 8]. The survival of one goat does not substantiate the usefulness of this drug combination for the treatment of *mastitis gravis* in small ruminants, but indicates the need of further trails.

RECOMMENDATION

The results of this trail suggest the beneficial employment of either sulphamonomethoxine, penicillin or tarantula poison for the treatment of *pododermatitis circumspecta* of cattle which have not suffered from hoof inflammation on a previous occasion. Theranekron is not registered in South Africa. It would offer an advantage over the other two drugs, should registration be achieved here without a withdrawal period, as in Germany. The less favourable results with cows suffering repeatedly from hoof inflammations rather suggest a surgical approach. Whether hoof surgery can be facilitated by previous treatment with tarantula poison, warrants further examination.

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ABSTRACT

SAMEVATTING

FOOT-AND-MOUTH DISEASE AND THE AFRICAN BUFFALO *(SYNCERUS CAFFER).* II. VIRUS EXCRETION AND TRANSMISSION DURING ACUTE INFECTION

Three groups of young buffalo in captivity were infected by exposing them to similar buffalo in the acute stages of infection induced by needle inoculation with SAT 1 or 2 viruses. Clear foot lesions developed in most of the buffalo from which the relevant virus types were re-isolated. During the first week following infection virus was found in blood, nasal secretions, saliva, preputial secretions and faeces. Air samples collected in the immediate vicinity of acutely infected buffalo were also found to contain virus. However, the regularity of virus detection as well as the quantity of virus in buffalo specimens was generally lower than for cattle infected with viruses of the same type. Conversely, virus was detected in the nasal secretions or saliva of 3 buffalo up to 4 weeks after infection, a situation which has not been encountered in cattle.

Susceptible cattle and impala *(Aepyceros melampus)* were penned together with or in the immediate vicinity of infected buffalo and shared feeding and watering facilities with the buffalo. The pattern of transmission which emerged indicated that transfer of these viruses from buffalo to other species occurs only in the acute stages of infection and where there is direct physical contact between the species. (Gainaru, M.D., Thomson, G.R., R.G., Esterhuysen, J.J., Bruce, W. & Pini, A., 1986. Foot-and-mouth disease in the African buffalo *(Syncerus caffer).* II. Virus excretion and transmission during acute infection. *Onderstepoort Journal of Veterinary Research,* 53, 75-85 (1986).)

ARTICLE ARTIKEL

PATHOGENESIS OF SUBCLINICAL BOVINE MASTITIS: PERSISTENCE, DETERIORATION AND IMPROVEMENT OF VARIOUS SUBCLINICAL CONDITIONS MONITORED BY MEANS OF THE INTERNATIONAL DAIRY FEDERATION/BOVINE SERUM ALBUMIN CRITERIA

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ABSTRACT: Giesecke W.H.; Barnard Marie-L. **Pathogenesis of subclinical bovine mastitis: Persistence, deterioration and improvement of various subclinical conditions monitored by means of the International Dairy Federation/Bovine Serum Albumin criteria.** *Journal of the South African Veterinary Association* (1986) 57 No. 2, 95-101 (En) Veterinary Research Institute, 0110 P.O. Onderstepoort, Republic of South Africa.

From this investigation, it is apparent that owing to the superior diagnostic differentiating of udder conditions by means of the IDF/BSA criteria, a range of detailed data has become available which facilitates an unprecedented insight in the dynamic balancing during normal lactation of several health states determinable at subclinical levels. The findings therefore from different points of view augment the corresponding results generated during the preceding investigation by means of the IDF criteria of acknowledged diagnostic limitations. In general, the data suggests that regardless of the diagnostic technique used for determinations during normal lactation on bovine udder conditions, udder health as such depends on 3 major determinants, namely intramammary epithelial integrity, intramammary somatic cellular defence and intrammamary bacterial challenge. Subclinical septic mastitis, clearly, develops through different types of gradually escalating deteriorations of health states which depend on several pathways of pathogenetic development. These are subject on the one hand to the 3 major determinants of udder health, and on the other hand to the nature and magnitude of their single and combined challenging. The deterioration of udder health in some quarters is opposed by the improvement of that in others which in the light of data on the rather transient persistence of health states implies, that deterioration of condition is at all levels of its development opposed directly but, possibly, somewhat delayed by the cow's efforts at restoring udder health as such to its completely normal level. All such changes depend on the dynamic balance between deteriorating, persisting and improving conditions that are by far more complicated than possibly conceivable from limited diagnositic differentiations facilitated by means of techniques which, in contrast to the triple-parameter determinations by means of the IDF/BSA criteria, depend on double-parameter and, still worse, on single-parameter determinations only.

Key words: Subclinical bovine mastitis, international dairy federation criteria, bovine serum albumin criteria.

INTRODUCTION

The data already presented[6] made it clear that the bovine udder, though not defenceless[9], nevertheless is exquisitely sensitive to bacterial challenge. It has further become evident that udder health generally depends on factors which may promote in the udder quarters the persistence, deterioration and improvement of health states, so that under circumstances facilitating almost natural (i.e. at random) bacterial challenging there is a balance of 2 major opposing trends, namely, one which through deterioration of health may lead to the subclinical manifestation of disease, i.e. mastitis, and the other one which through restoration of health may permit the manifestation and maintenance of normal udder health. These 2 major trends between the 2 poles at the extremes of udder health apparently criss-cross each other at 2 different levels of udder health determinable by means of the International Dairy Federation (IDF) criteria as so-called latent infection (LI) and aseptic mastitis (AM)[10].

Although such developments seem clear in principle, the results pointed out above nevertheless have indicated certain inconsistencies: (i) As conditions intermediate to the normal and mastitic states, as the 2 extremes of udder health, LI and AM strangely enough tend neither particularly towards one nor the other of these extreme states, but seem to be almost equally important to both of them; (ii) LI is related to lacteal levels of somatic cells and Bovine Serum Albumin (BSA), and AM to bacteriological findings and lacteal levels of BSA which are completely inconsistent if, as the terms LI and AM imply, the former amounts to a type of trully intramammary albeit latent infection, and the latter to some type of trully intramammary albeit unspecific inflammation in an organ of acknowledged high sensitivity to lesions from bacterial challenge and other agents.

Because of such inconsistencies, further doubts expressed on the concept as such of latent udder infections[8], several major diagnostic limitations of the IDF criteria[4] for routine investigations and considerable differences of lacteal concentrations of glucose in milk from quarters with different health conditions[5], it was prudent not only to assess samples of milk depending on the double-parameter IDF criteria[10] but also the triple-parameter IDF/BSA criteria[4]. Results from the former evaluations have already been discussed[6] whereas findings from the latter determinations are presented below.

MATERIALS AND METHODS

The general experimental conditions of the preceding research[6] and this investigation conducted in parallel, were identical except that during this one the double-parameter determinations by means of the IDF criteria[10], were augmented with BSA evaluations. Therefore, udder conditions were diagnosed depending on triple-parameter determinations by means of the IDF/BSA criteria already described in principle

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Table 1: Proposed key for the differentiation between subclinical mastitis and other udder health states in normally lactating cows by means of SCC, bacteriological and BSA determinations

SCC values x10³ per ml of milk	Culture of potentially pathogenic bacteria	Diagnosis depending on IDF criteria[10]	BSA values (diameter of precipitation zone in mm)	Diagnoses depending on IDF/BSA criteria [4]*
≤ 500	Negative	Normal secretion (N)+	<8,0 ≥ 8,0	Completely normal (CN)+ Unspecific hyperalbumingalactia (UHAG)
>500	Negative	Non-specific mastitis (AM)	<8,0 ≥ 8,0	Unspecific cellular reaction (UCR) Aseptic mastitis (AM)
≤ 500	Positive	Latent infection (LI)	<8,0 ≥ 8,0	Irrelevant teat canal infection (ITI) Specific hyperalbumingalactia (SHAG)
>500	Positive	Mastitis (M)	<8,0 ≥ 8,0	Relevant teat canal infection (RTI) Septic mastitis (SM)

*Amended depending on additional data[2,3];
+ abbreviations used below

elsewhere[4], but slightly amended after due consideration of more recent findings[2,3], which clearly indicate that the conditions A and B pointed out previously[2,3,4], may be termed unspecific (U) and specific (S) hyperalbumingalactia (HAG) (Table 1).

With regard to the proposed diagnostic key (Table 1), it may be pointed out that the 4 conditions depending on cytological and bacteriological evaluations recommended by the IDF[10] are further subdivided into 2 health states each, depending on normal and abnormal lacteal levels of BSA determined and interpreted as already discussed elsewhere[4].

Table 2: General prevalence, diagnostic and other characteristics of 8 udder conditions determined by means of the IDF/BSA criteria

Designations		Udder health states x values							
		SM***	RTI	SHAG	ITI	AM	UCR	UHAG	CN
General prevalence of conditions (numbers of quarters involved)		100	341	8	311	9	85	28	944
Pathogenic bacteria in milk samples collected aseptically		Present	Present	Present	Present	Absent	Absent	Absent	Absent
$\bar{x}$ ± SD* values of SCCx10³/ml of milk		4233 ±3352	2138 ±2072	214 ±149	167 ±135	3744 ±3183	3975 ±4204	86 ±94	83 ±83
$\bar{x}$ ± SD values of BSA determinations		9,42 ±2,12	6,45 ±0,78	8,53 ±0,68	6,09 ±0,85	8,69 ±0,70	6,26 ±0,75	8,27 ±0,42	5,95 ±0,80
$\bar{x}$-persistence (days) of bacteriologically and otherwise diagnostically identical states of ud-health**		2,02	2,97	1,0	2,81	1,0	1,51	1,12	6,83
Corresponding diagnosis by means of the IDF criteria	Conditions	M		LI		AM		N	
	General prevalence	441		319		94		972	

*Mean ± standard deviation;
**N.B. Change from one to another health state does not necessarily mean that an affected quarter becomes completely normal and *vice versa*, nor does it preclude recurrence of the initial condition
***See Table 1 for explanation of abbreviations

RESULTS

Range, diagnostic and dynamic characteristics of udder conditions on the subclinical level

The investigation, using the cytological, bacteriological and BSA parameters and criteria of the diagnostic key indicated (Table 1), showed 8 udder conditions which differed in their general prevalence, diagnostic and supplementary characteristics (Table 2).

As regards the 4 udder conditions determined by means of the IDF criteria (Table 2) and further differentiated into 2 health states each by means of the IDF/BSA criteria (Table 2), it is evident that the 4 pairs of conditions thus diagnosed have 1 major characteristic in common not recognizable by means of the IDF criteria, namely, one health state each with mean values of BSA in the mastitic range and another health state each with mean values of BSA in the non-mastitic to completely normal range. The former clearly indicates abnormally elevated intramammary epithelial permeability to BSA, whereas the latter suggests that such permeability varies within ranges normal to almost normal.

It is therefore evident that each of the different health states is associated with corresponding changes at the level of intramammary epithelial integrity.

From the cytological and bacteriological findings (Table 2) it is equally apparent that each of the different health states further is associated with corresponding changes at the level of intramammary somatic cellular defence and the level of intramammary bacterial challenge.

Collectively, all such changes at the 3 different major levels of paramount signifance to the general functioning of the lactating bovine udder make it obvious, therefore, that regardless of the health condition diagnosed during normal lactation, udder health as such depends on 3 major determinants, namely, intramammary epithelial integrity, somatic cellular defence and bacterial challenge.

The 8 health states differentiated by means of the IDF/BSA criteria apparently depend on factors affecting these 3 major determinants of bovine udder health either singly or in different double- and triple-combinations. The health states *UHAG*, *UCR* and *ITI* each seem to depend on changes limited to a single but different one of the 3 major determinants, namely, epithelial integrity, somatic cellular defence and, bacterial challenge, respectively. As a class of conditions, they may thus be considered that class representing the level of single-determinant challenge (i.e. conditions simply referred to below as Class 1 conditions). In contrast, the health states SHAG, AM and *RTI* each seem related to changes of different double-combinations of the 3 major determinants, such as epithelial integrity/bacterial challenge, epithelial integrity/somatic cellular defence and somatic cellular defence/bacterial challenge, respectively. As a class of conditions, they may thus be considered that class representing the level of double-determinant challenge (i.e. conditions simply referred to below as Class 2 conditions). The conditions SM and CN, as opposite poles at the extremes of udder health on subclinical levels, are each characterized, in turn, by the presence and absence of concurrent changes of the same triple-combination of the 3 major determinants at the basis of bovine udder health.

Dynamic fluctuations of udder conditions during periods of 24 h each

The results above have shown that the augmentation of the IDF criteria with the BSA determinations doubled the range of health states differentiated. The findings on each of these 8 conditions (Table 2), singly and collectively, provided new insight on their characteristics and collective significance to bovine udder health as such.

However, as important as the doubling of diagnostic differentiations by means of the IDF/BSA criteria may be from the point of view of the characteristics of the health states determined, it had still greater implications on the monitoring of dynamic fluctuations of such conditions, for it facilitated a quadrupling of data generated on the dynamics of subclinical mastitis proper and several related conditions (Table 3a).

Table 3a: Distribution of conditions determined by means of the IDF/BSA criteria on 1743** quarters monitored

Udder conditions (UC) and distribution of quarters		Udder conditions and redistribution of quarters during 24 h intervals between the initial and final investigations of the 21 consecutive UHMHE's**							
At start of 24 h intervals		SM	RTI	ITI	SHAG	AM	UCR	UHAG	CN
UC	Number of quarters								
SM***	98	50*	30	9	3	2	1	—	3
RTI	327	30	228*	46	—	2	11	—	10
ITI	293	11	42	196*	—	1	5	4	34
SHAG	8	3	2	2	1*	—	—	—	—
AM	8	1	2	2	—	—*	3	—	—
UCR	79	—	16	6	2	—	22*	3	30
UHAG	27	—	2	2	—	—	3	—*	20
CN	903	—	7	34	1	2	28	20	881*
At conclusion of 24 h intervals: Numbers of quarters		95	329	297	7	7	73	27	908

*Quarters with udder conditions persisting unchanged during a 24 h period. N.B.: Quarters redistributing above and below diagonal line of *indicate improving and deteriorating conditions

**For explanation of difference between 1826 quarters investigated and 1743 quarters monitored, during the udder health monitoring herd examinations (= UHME-s), see[6]

***See Table 1 for explanation of abbreviations.

The data (Table 3a) indicate 5 major groups of results, namely, the initial and final point prevalence of each health state and corresponding dynamic fluctuations depending on the persistence, deterioration and improvement of condition.

For example, quarters persistently CN (Table 3a) suggest that the cows successfully maintained completely normal health in 811 out of 903 udder quarters CN at the initial investigations. However, normal udder health was not successfully maintained in al of those 903 quarters (Table 3b)

From the data (Table 3b) it is apparent that out of the 903 quarters initially CN 0,78%; 3,77%; 0,11%; 0,22%; 3,10% and 2,21% developed RTI, ITI, SHAG, AM, UCR and UHAG.

From the point of view of the initial point prevalence of CN, such percentage values are rather limited. However, owing to the large number of CN initially involved, each of these values nevertheless became important from the point of view of the final point prevalence of most of the health states monitored (Table 3c).

For example, the 20 new cases of UHAG (Table 3a) only amounted to 2,21% of the 903 quarters initially CN (Table 3b), but to 74,07% of the quarters affected with UHAG at termination of the 24 h intervals (Table 3c).

During the 24 h periods between initial and final UHMHE's (Table 3a, b, c), a wide range of changes similar in principle to those affecting quarters initially CN, also occurred in quarters initially affected with SM, RTI, ITI, SHAG, AM, UCR and UHAG. From all such fluctuations it is evident that the different health states determined initially persisted unchanged in 75,05% of quarters, whereas they changed to conditions suggesting the improvement and deterioration of health in 12,44% and 12,51%, respectively, of the 1743 quarters monitor-

Table 3b: Distribution and corresponding redistributions per initial point prevalence of each health state determined by means of the IDF/BSA criteria on the 1743 quarters monitored*

Udder conditions (UC) and distributions of quarters		Relative redistribution of quarters during 24 h intervals between the initial and final investigations of the 21 consecutive UHMHE's calculated where IPP values = 100% each of corresponding udder condition							
At start of 24 h intervals (= initial point prevalence = IPP):		%IPP							
UC	Numbers of quarters	SM	RTI	ITI	SHAG	AM	UCR	UHAG	CN
SM***	98 (=100%)	51,02*	30,62	9,18	3,06	2,04	1,02	—	3,06
RTI	327 (=100%)	9,18	69,73*	14,07	—	0,61	3,36	—	3,06
ITI	293 (=100%)	3,75	14,34	66,89*	—	0,34	1,71	1,37	11,60
SHAG	8 (=100%)	37,5	25,00	25,00	12,50*	—	—	—	—
AM	8 (=100%)	12,50	25,00	25,00	—	—*	37,50	—	—
UCR	79 (=100%)	—	20,25	7,60	2,35	—	27,85*	3,80	37,98
UHAG	27 (=100%)	—	7,41	7,41	—	—	11,11	—*	74,07
CN	903 (=100%)	—	0,78	3,77	0,11	0,22	3,10	2,21	89,81
At termination of 24 h intervals Numbers of quarters		(95)	(329)	(297)	(7)	(7)	(73)	(27)	(908)

* For further detail see Table 3a
** See Table 1 for explanation of abbreviations

Table 3c: Distribution and corresponding redistributions per final point prevalence of each health state determined by means of the IDF/BSA criteria on the 1743 quarters monitored*

Udder conditions (UC) and distributions of quarters		Relative redistribution of quarters during 24 h intervals between the initial and final investigations of the 21 consecutive UHMHE's calculated where FPP values = 100% each of corresponding udder condition							
At start of 24 h intervals (= initial point prevalence = IPP):		%FPP							
UC	Numbers of quarters	SM	RTI	ITI	SHAG	AM	UCR	UHAG	CN
SM***	(98)	52,63*	9,11	3,03	42,85	28,57	1,37	—	3,33
RTI	(327)	31,58	69,30*	15,49	—	28,57	15,07	—	1,10
ITI	(293)	11,58	12,77	65,99*	—	14,29	6,85	14,81	3,75
SHAG	(8)	3,16	0,61	0,67	14,29*	—	—	—	—
AM	(8)	1,05	0,61	0,67	—	—*	4,11	—	—
UCR	(79)	—	4,86	2,02	28,57	—	30,14*	11,11	3,30
UHAG	(27)	—	0,61	0,67	—	—	4,11	—*	2,02
CN	(903)	—	2,13	11,46	14,29	28,57	38,35	74,07	89,32*
At termination of 24 h intervals Numbers of quarters		95 (95)	329 (329)	297 (297)	7 (7)	7 (7)	73 (73)	27 (27)	908 (908)

* For further detail see Table 3a
** See Table 1 for explanation of abbreviations

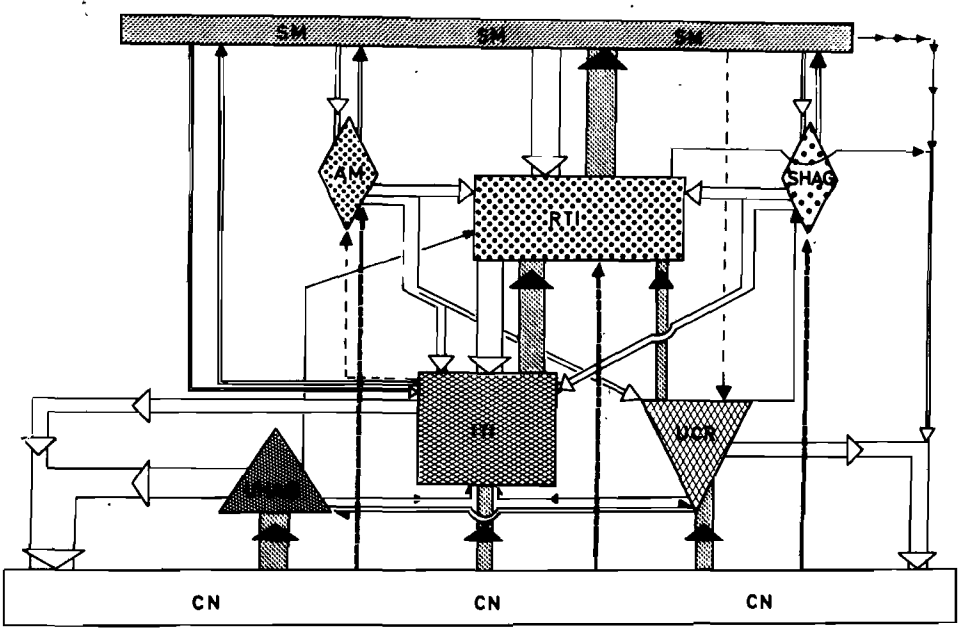

Fig. 1: Schematic presentation of the dynamic balance during 24 h periods of 8 interdependent subclinical health states diagnosed by means of the triple-parameter IDF/BSA criteria and represented by the corresponding pools of quarters with SM (shaded area), N (unshaded area), Class 1 conditions (meshed areas) of UHAG (fine mesh), ITI (middle mesh) and UCR (coarse mesh) and Class 2 conditions (dotted areas) of AM (small dots), RTI (medium dots), SHAG (large dots), each subject to fluctuations of persistent (areas), improving (unshaded arrows) and deteriorating (shaded arrows) udder conditions. The arrows indicate the dynamic changes of conditions along certain pathways apparently related to the deterioration and restoration of bovine udder health as suggested by results in Table 3 above.

ed (Table 3a).

These general findings make it clear that udder health of the small herd monitored, depended on a core of quarters where SM and CN, as extreme states, and RTI, ITI, SHAG and UCR, as intermediate states of the scale of conditions, persisted for some albeit rather limited time (Table 2). However, udder health further depended on 2 major types of developments opposite in nature but almost identical in magnitude, namely, the improvement of condition in some quarters and the deterioration of condition in other quarters possibly of the same udder.

The individual health states differed in their significance to the dynamic balance as such of udder health states depending on their tendencies to persist, improve and deteriorate (Fig. 1).

The Class 1 conditions of UHAG, UCR and ITI (Fig. 1) were associated with pools of quarters (Table 3a) generally showing a mutual exchange of quarters particularly active (= 166 quarters exchanged) with the pool of CN quarters, limited (= 23 quarters exchanged) amongst the conditions at the same level of single-determinant challenging and intermediate (= 127 quarters exchanged) with pools of quarters affected with RTI, SHAG and AM, i.e. Class 2 conditions at the level of double-determinant chalenging. Sixty of the 101 new cases of RTI (Table 3a) came from the pools of quarters with ITI (=42 cases), UCR (= 16 cases) and UHAG (=2 cases). The pool of quarters with ITI was 4 x more important as source of new cases of RTI (=42 cases) than of SM (=11 cases).

The Class 2 conditions of RTI, SHAG and AM (Fig. 1) were associated with pools of quarters (Table 3a) generally showing a mutual exchange of quarters particularly active (=127 quarters exchanged) with pools of ITI, UCR and UHAG quarters, intermediate (= 69 quarters exchanged) with the pool of SM quarters and limited (=7 quarters exchanged) amongst pools of quarters affected with RTI, SHAG and AM, i.e. conditions at the same level of double-determinant challenging. Thirty-four of the 45 new cases of SM (Table 3a) came from the pools of quarters with RTI (=30 cases), SHAG (= 3 cases) and AM (=1 case). The pool of quarters with RTI was 1,5 x more important as source of new cases of ITI (=46 cases) than of SM (=30 cases). Proportionately (Table 3b), quarters initially affected with SHAG newly developed SM 3 x, 4 x and 10 x more readily than quarters initially affected with AM, RTI and ITI, respectively.

From all the above-mentioned findings it is clear that new cases of SM did not develop directly from quarters initially CN. That development amounted, instead, to a more gradual deterioration of udder health. Starting at the level of complete normality to eventually terminating at the level of subclinical disease manifestation, the deterioration of condition advanced through levels of single- and/or doubledeterminant challenging dependent on pathways of pathogenetic development presumably determined on the one hand by the 3 major determinants of udder health and, on the other hand, by the nature and magnitude of their single and combined challenge.

The deteriotation of udder health in some quarters was opposed by the improvement of that in others. Restoration of completely normal condition was most limited at the level of subclinical disease manifestation

(Table 3c) and became 3,3 x and 28 x more effective at the levels of double- and single-determinant challenging, respectively.

The above data on deterioration and improvement of udder health, as the 2 opposing major tendencies of the dynamic balance of subclinical udder conditions, last but not least, suggest clearly that under the conditions of this investigation one may consider the natural ability of the cows to maintain normal udder health, probably the most effective measure available against the development during normal lactation of subclinical septic mastitis proper and several related precursor-like conditions.

DISCUSSION

This investigation, based upon determinations by means of the IDF/BSA criteria (Table 1), has confirmed the findings from the preceding investigation[6] on the general persistence, deterioration and improvement of udder health states monitored by means of the IDF criteria. However, because of several acknowledged diagnostic limitations of the latter criteria[1,4,7], it further has become obvious that results determined by means of the IDF and IDF/BSA criteria may significantly differ in the detail of findings on several aspects, such as range, general prevalence, initial and final point prevalence, diagnostic characteristics and dynamic balancing of udder health states. Such differences depend on the superior diagnostic differentiation of subclinical udder conditions facilitated by means of the IDF/BSA criteria suggested earlier[4] and in the meantime verified directly and indirectly by a considerable number of different workers.

As regards the range, general prevalence and characteristics of health states it is apparent from data above (Table 2) that the 4 health states diagnosed by means of the IDF criteria may be further differentiated into 2 completely different conditions each by means of the IDF/BSA criteria (Table 1). Of course, one may hold that such differentiations are not justified because of the large standard deviation of the SCC and BSA values. However, that argument is not necessarily valid for the following reasons: (i) On the subclinical level functional changes of the lactating mammary gland usually amount to subtle fluctuations escalating gradient-like to eventually differ at certain stages more by degrees than by clear-cut specific normal-abnormal parameters; (ii) The differentiation of health states by means of the IDF/BSA criteria is by far more justifiable than that by means of the IDF criteria if judged in the light of available conclusive data on teat canal infections and intramammary epithelial lesions truly occurring [1,4,7] but not diagnosed by means of the IDF criteria; (iii) The conditions SM, SHAG, AM and UHAG show BSA mean values clearly different from those related to the conditions RTI, ITI UCR and CN (Table 2); (iv) Diagnostic differences of the conditions differentiated by means of the IDF/BSA criteria are not limited to those pointed out above (Table 2) and similar ones described elsewhere[1,4] but further include differences of lacteal levels of glucose[5] and of nucleases of which related to SM clearly differ from those associated with TI[7]; (v) Contrary to determinations by means of the IDF criteria those by means of the IDF/BSA criteria are consistent with extensive and conclusive histological, histopathological, and other research findings indicating that udder health, clearly depends on intramammary epithelial integrity, somatic cellular defence and bacterial challenge.

The differentiation of the 8 conditions by means of the IDF/BSA criteria has major implications for the monitoring and interpreting of dynamic fluctuations affecting during normal lactation bovine udder health at the subclinical level. It thus has become evident from the 8 conditions differentiated by means of the IDF/BSA criteria that udder health depends on 3 major determinants, namely, intramammary epithelial integrity, somatic cellular defence and bacterial challenge. It has further become apparent that the health states UHAG, UCR and ITI at the level of single-determinant challenging and the states SHAG, AM and RTI at the level of double-determinant challenging are all conditions intermediate to the 2 extreme conditions at the opposite poles of udder health, namely SM at the level of subclinical disease manifestation and CN at the level of complete normality. The detailed findings on dynamic fluctuations of each of these conditions (Table 3, Fig. 1) make it further abundantly clear that the dynamic balance as such of udder health states depends on their respective tendencies to persist, improve and deteriorate. From the point of view of practical work on subclinical mastitis it is especially noteworthy that at the subclinical level all udder health states are rather labile (Table 2). This has important implications for the routine diagnosis, therapy, control and prevention of the disease.

During this investigation subclinical septic mastitis (SM) did not develop directly from quarters initially completely normal. Its development apparently amounted, instead, to a gradual deterioration of udder health that advanced through the levels of single-and/or double-determinant challenging depend upon pathways of pathogenetic development presumably determined on the one hand by the 3 major determinants of udder health and on the other hand by the nature and magnitude of their single and combined challenging. It therefore seems conceivable that further research by means of the IDF/BSA and similar combinations of other criteria could be very advantageous to a more complete understanding of various problems to the pathogenesis of and natural defence against subclinical types of mastitis.

The most effective measure available against the development during this investigation of SM and several related precursor-like conditions, was the natural ability of the cows to maintain udder health completely normal.

ACKNOWLEDGEMENTS

Advice and assistance of Dr H van Ark (Department of Agriculture and Water Supply, Division of Datametric Services) on statistical aspects of the investigation are gratefully acknowledged.

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ABSTRACT

SAMEVATTING

THE RELATION OF CLIMATE AND TOPOGRAPHY TO GASTRO-INTESTINAL NEMATODE WORM EGG COUNTS OF ANGORA GOATS IN THE EASTERN CAPE

Haemonchus, Trichostrongylus, Ostertagia and *Nematodirus* worm populations of Angora goats, based on differential egg counts, are considered in relation to climatological and topographical data. Egg counts indicated that the estimated worm populations in goats that experienced wet circumstances were higher than those exposed to dry conditions. Wetness was assessed by relating spring, summer, and early autumn rainfalls to ground slope. It is proposed, that tactical anthelmintic treatments of goats be based on the degree of wetness of the grazing of property. (Mcculloch, B., Dalbock, R. R. & Kühn, H G., 1986. The relation of climate and topography to gastro-intestinal nematode worm egg counts of Angora goats in the Eastern Cape. *Onderstepoort Journal of Veterinary Research,* 53, 167-177 (1986).)

ABSTRACT

SAMEVATTING

THE INABILITY OF A SOUTH AFRICAN *BABESIA BOVIS* VACCINE STRAIN TO INFECT *BOOPHILUS MICROPLUS*

A strain of *Babesia bovis* that had been attenuated by rapid syringe passage through a series of 23 splenectomized calves was unable to infect its vector *Boophilus microplus*. An attempt to transmit the attenuated Australian *Babesia bigemina* G strain with a South African strain of *B. microplus* was likewise unsuccessful. The epidemiological implication of these observations in terms of babesiosis control is discussed. (Mason, T. E., Potgieter, F.T. & Van Rensburg, L., 1986. The inability of a South African *Babesia bovis* vaccine strain to infect *Boophilus microplus. Onderstepoort Journal of Veterinary Research,* 53, 143-145 (1986).)

ABSTRACT

SAMEVATTING

AFRICAN SWINE FEVER. I. MORPHOLOGICAL CHANGES AND VIRUS REPLICATION IN BLOOD PLATELETS OF PIGS INFECTED WITH VIRULENT HAEMADSORBING AND NON-HAEMADSORBING ISOLATES

Replicating and mature viral particles were detected with the transmission electron microscope in blood platelets of pigs infected with virulent haemadsorbing and non-heamadsorbing African swine fever virus isolates. Although platelet numbers decreased terminally in infected pigs, the most noticeable morphological damage to these cells apparent in the last 2 days of the disease included cytoplasmic swelling, vacuolation, fragmentation and loss of dense granules. (Neser, J.A., Phillips, T., Thomson, G. R., Gainaru, M. D. & Coetzee, T., 1986. African swine fever. I. Morphological changes and virus replication in blood platelets of pigs infected with virulent haemadsorbing and non-haemadsorbing isolates. *Onderstepoort Journal of Veterinary Research,* 53, 133-141 (1986).)

OXYTETRACYCLINE CONCENTRATIONS IN PLASMA AND SEMEN OF RAMS

A. IMMELMAN and GILIAN DREYER*

ABSTRACT: Immelman A.; Dreyer Gilian. **Oxytetracycline concentrations in plasma and semen of rams.** *Journal of the South African Veterinary Association* (1986) 57 No. 2, 103-104 (En) Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

The concentration of oxytetracycline in plasma and semen of mature rams was determined after intravenous administration. Therapeutic concentrations were attained in semen but were not maintained for as long as in the plasma.

Key words: Oxytetracycline, plasma, semen, sheep.

INTRODUCTION

Oxytetracycline is the form of tetracycline most often used in South Africa. It is usually given intramuscularly but can be given via other routes. This is a broad-spectrum antibiotic with efficacy against numerous Gram+ and Gram− bacteria. Certain rickettsiae, mycoplasma and protozoa species will also be affected by oxytetracycline. The effect on these micro-organisms is the blocking of protein synthesis in the rapidly growing and reproducing cells. The antibiotic is classified as a bacteriostatic agent but in high concentrations it may become bactericidal[2].

The use of oxytetracyline in the treatment of orchitis in rams is not well documented but it is used in the clinical field to treat suspected bacterial infections. It may be expected that oxytetracycline will be effective in these cases because this antibiotic disperses throughout the body, attaining therapeutic levels in most tissues and fluids within a short time. A concentration of 0,5 to 1 μg/ml has been accepted as adequate for most purposes[1].

The purpose of this study was to determine the concentration of oxytetracycline in semen after a single intravenous administration of an oxytetracycline solution.

MATERIALS AND METHODS

As experimental animals, 4 adult Merino rams were used. These animals were housed indoors in individual pens and were fed chopped lucerne on an ad lib basis.

The formulation of oxytetracycline used for this study was a commercially available preparation with a concentration of 100 mg/ml using propylene glycol as a vehicle (Liquamycin, Pfizer Laboratories). This drug was administered intravenously into the *vena jugularis* at a dose of 10 mg/kg live body mass. The full dose was given over a period of 30 seconds. Blood samples for the determination of the plasma concentrations were collected from the opposite *vena jugularis*. Samples were collected in heparin at 1, 2, 4, 6, 8 and 10 hours after administration. After centrifugation the plasma was collected and stored at −20°C until the analyses were done.

Semen specimens were collected by the use of electrical stimulation through a probe in the rectum. An ejaculate of 0,5-1 ml was considered to be sufficient for analytical purposes. Each sample was immediately examined for colour, density and quality of sperm. This was done to ensure that semen and not only secretions from accessory glands were present in the ejaculate. All specimens were collected simultaneously with the blood samples and stored at -20°C until the analyses were performed.

A standard microbiological method was used to determine the oxytetracycline concentrations in both semen and plasma. Peptone agar was prepared as described by the manufacturer and the pH adjusted to 6 by adding hydrochloric acid. Spores of *Bacillus cereus* were mixed into the agar after the solutions were allowed to cool down but before it started solidifying. The agar was then poured into glass dishes making sure that the thickness of the layer was absolutely even. The concentration of the spores was predetermined to give just sufficient growth to be able to determine a clear line of growth inhibition.

After solidification, wells were punched into the agar using a punch with a diameter of 5mm. Into each well sufficient test solution was placed to fill the well completely. Care was taken not to overflow the well, but enough solution had to be put in so that fluid remained in the well after incubation. The plates were placed in an incubator at 29°C for 16 hours. The diameter of the zones of bacterial growth inhibition around each well was measured. These results were plotted on 2 cycle semilogarithmic paper giving the concentration of the standard solutions in microgram per millilitre on the x-axis and the diameter of the zone of inhibition on the y-axis.

Standard solutions of oxytetracycline were prepared in normal sheep's plasma varying in concentrations from 0,5 − 12 μg/ml. The same concentrations were also prepared in normal ram's semen. These solutions were then used to set up the standard curves for the samples to be analysed. The samples for the standard curves and the collected plasma and semen specimens were placed on the same agar plates. The sensitivity of this microbiological system was established to be 0,5 μg/ml. The concentrations of the unknown specimens were determined from the standard curve after measuring the zones of inhibition.

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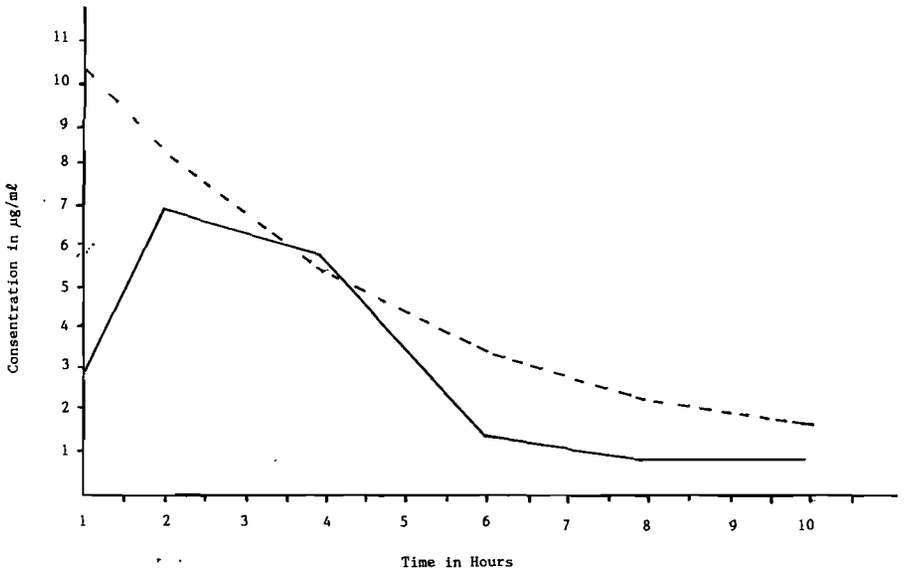

Fig. 1: The concentration of oxytetracycline in plasma and semen of rams after intravenous administration. Plasma ..... Semen _____

RESULTS AND DISCUSSION

The frequent collection of small volumes of semen from the rams did not affect the quality of the semen. Density and motility remained within the normal limits although the quality from the various collections of an individual fluctuated within these limits.

The concentration of oxytetracycline in the plasma and semen at the different time intervals are tabulated in Table 1. The mean and the standard deviation for each collection are given. The mean values are also represented in Figure 1.

Table 1. The concentration of oxytetracycline in plasma and ram's semen after intravenous administration. (Mean ± standard deviation (S.D.); n = 4).

Sample	Plasma		Semen	
Time of collection	Mean	S.D.	Mean	S.D.
1 hour	10,5	2,2	3,0	5,2
2 hours	8,4	1,6	7,0	4,6
4 hours	5,5	1,3	5,9	4,0
6 hours	3,5	1,2	1,4	0,45
8 hours	2,4	0,79	0,8	0,4
10 hours	1,78	0,7	0,9	0,4

After an intravenous injection the very high concentration observed in the plasma after 1 hour, is to be expected. The concentration in the semen at that stage, was only about one third of the plasma concentration. Both concentrations were, however, far above the therapeutic requirements (0,5-1 µg/ml).

The maximum level of oxytetracycline in the semen was reached two hours after administration. The decline of oxytetracycline concentration in the semen appears to be more rapid than in the plasma. The therapeutic concentrations of 1 µg/ml was maintained in the semen for only 7,25 hours, while the plasma concentration was far above this level when the experiment was terminated after 10 hours. Both levels, however, remained above 0,5 µg/ml which could have therapeutic value.

From these results it cannot be determined where the oxytetracycline in the semen originated from. This could have come from the testicular tissue or could have been excreted with the secretions from the accessory glands. Further investigation will have to be done to clarify this point.

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RESEARCH NOTE **NAVORSINGSVERSLAG**

ADVERSE EFFECTS FOLLOWING INTRAVENOUS FLUID THERAPY IN THE HORSE USING NON-COMMERCIAL FLUIDS: PRELIMINARY FINDINGS

M. DENKHAUS and S. VAN AMSTEL*

ABSTRACT: Denkhaus M.; Van Amstel S. **Adverse effects following intravenous fluid therapy in the horse using non-commercial fluids: Preliminary findings.** *Journal of the South African Veterinary Association* (1986) 57 No. 2, 105-107 (En). Department of Medicine, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

Non-commercial, endotoxin positive, intravenous fluids as well as a commercially available intravenous fluid were given to clinically normal horses. Endotoxin-positive fluids caused clinical signs attributable to endotoxaemia. Leukopenia, preceded by a fluctuating white cell count, was observed in horses showing clinical signs. The commercial intravenous fluid had no effect on the white cell count or on the clinical state. Precautions to be taken and recommendations are made with regard to the monitoring of horses in which one might be forced to use non-commercial intravenous fluids.

Key words: Horse, non-commercial intravenous fluids, fluid therapy, endotoxins, *Limulus amebocyte* lysate gelation test.

INTRODUCTION

Intravenous fluid therapy in the horse often involves the use of large volumes of polyionic solutions which incur high costs[4]. For this reason veterinarians may resort to using non-commercial intravenous solutions. It is, however, difficult to control the sterility of such solutions and the containers used for their administration[7]. Even low levels of endotoxins in such solutions, may be sufficient to cause endotoxaemic signs in the infused animal when large enough volumes are infused[7]. A sublethal dose of endotoxins classically causes pyrexia, sweating, diarrhoea, muscle tremors, hyperpnoea and a dramatic and sudden leukopenia[2,5,8].

The aim of this study was to examine the clinical and leukocyte response to the infusion of non-commercial solutions in normal horses. The presence of endotoxins in such solutions was also determined.

MATERIALS AND METHODS

Animals:

Three groups (A, B and C) of three horses each, were used. In all groups one horse was starved and voluntary water consumption prevented for the duration of the experiment. This was done in an attempt to simulate the lack of water and feed intake in an anorectic patient. The second and third horse in all groups were fed and watered ad lib. throughout. In each group the starved horse and one other were subjected to intravenous fluid therapy, with the remaining horse acting as control.

Fluids:

Fluid administration was performed as follows: an area over the right jugular vein was shaved and aseptically prepared and an intravenous catheter was placed into the vein. A maintenance fluid requirement of approximately 54 ml/kg/24 hours[12] was infused over an 8 hour period. The average weight of the horses was 400 kg, which implied a maintenance volume of 21,6 ℓ/24 hours. Due to practical considerations however, only 20 ℓ were infused in 8 hours. These 8 hour infusion periods were repeated on consecutive days for a maximum of 3 infusion periods, or until clinical signs of discomfort were seen. During each of these periods, blood in EDTA was collected from the left jugular vein. Blood was taken just before the infusions were begun each morning, 4 hours into the infusion period, and the last sample 10 minutes after the infusion was completed. Total leukocyte counts (WCC) were performed on each sample using an electronic cell counter (Coulter Counter Model Fn. Coulter Electronics Inc. Hialeah-Florida USA).

Groups A and B received an intravenous solution stored in 10 ℓ polyethylene plastic containers previously cleaned with a chlorhexidine-cetrimide (Savlon, ICI South Africa (Pharmaceuticals)) solution and subsequently rinsed with sterile water. The solution was made up to provide approximately 140 mmol/ℓ Na^+, 55 mmol/ℓ K^+, 115 mmol/ℓ Cl^- and 30 mmol/ℓ acetate (Pharmacy Handout for Seniors 1982, Fort Collins, Colorado State University, USA). In group A, deionised water was used to dissolve the salt mixture. In group B water obtained by reverse osmosis was used and in Group C a commercially available polyionic intravenous fluid, (Plasma Replacement Fluid, Sabax).

Experimental design

Two of the three horses in each of the experimental groups (A, B and C) received intravenously fluids while the third horse served as a control.

Fluid administered intravenously to horses in group A was made up using deionised water. For group B, water obtained by reverse osmosis was used while group C received a commercial fluid.

In all three groups one of the two horses receiving i/v fluids and the control were given food and water ad lib. while the remaining horse was starved and received no water.

Endotoxin determinations were carried out on the liquid fractions of the solutions made up with deionised water and water obtained with reverse osmosis as well as the already made up commercial solution. For this purpose a *Limulus amebocyte* lysate (LAL) gelation test as described by Levine & Bang[8] was used.

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RESULTS

Group A – Fed and watered horse receiving fluids:

This horse showed diarrhoea, sweating, pyrexia (rectal temperature: 39,9°C) and hyperpnoea, 2,5 hours after the start of the second infusion period.

During the first period, however, fluctuation in the WCC had already occurred: from a preinfusion level of 14,4 x $10^9/\ell$ it dropped to 3,2 x $10^9/\ell$ at the middle sampling. The WCC was high at the end of the first period and even higher at the beginning of the second period (16,6 x $10^9/\ell$ and 27,5 x $10^9/\ell$, respectively). At the onset of clinical signs the WCC was 2,4 x $10^9/\ell$. At this point the infusion was discontinued after which the WCC rose progressively to 14,4 x $10^9/\ell$ and returned to normal a few days later (Fig. 1).

Group A - Starved horse receiving fluids:

Clinical signs were seen in this horse 3 hours into the third period which included sweating, pyrexia (rectal temperature: 39°C), hyperpnoea and on auscultation of the abdomen, sounds indicating fluid-filled intestines, could be heard. Similar to the first animal, fluctuation in the WCC occurred during the first two infusion periods: from preinfusion levels of 8,9 x $10^9/\ell$ a progressive drop to 6,4 x $10^9/\ell$ occurred during the first period. At the beginning of the second infusion period the WCC was 18,9 x $10^9\ell$, but dropped to 15,6 x $10^9/\ell$ and rose to 19,5 x $10^9/\ell$ at the last sampling of that period. At the onset of the third period the WCC was 15,2 x $10^9/\ell$, but dropped to 1,3 x $10^9/\ell$ three hours later coinciding with the clinical signs as previously decribed (Fig. 1).

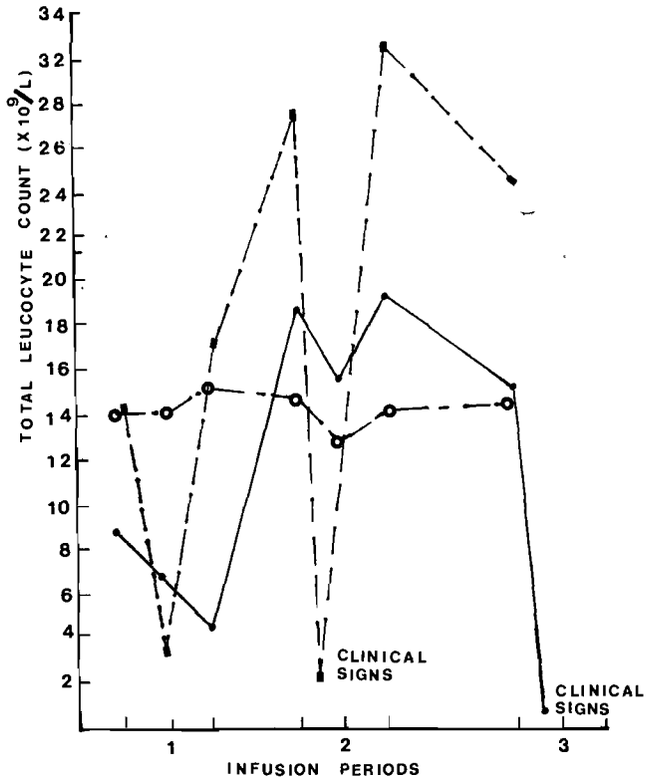

Fig. 1: ■— —■horse fed & watered ad lib. plus fluids
●——●horse starved plus fluids
○— —○control horse

Group B - Fed and watered horse receiving fluids:

The following clinical signs were seen 4 hours after the start of the second infusion period: mild pyrexia (rectal temperature: 38,8°C), intermittent bouts of sweating and muscle tremors. Again fluctuations in the WCC had occurred at each previous sampling: from preinfusion levels of 9,7 x $10^9/\ell$ it dropped to 8,3 x $10^9/\ell$ and rose to 13,2 x $10^9/\ell$ at the end of the first period. At the beginning of the second period the WCC was 9,6 x $10^9/\ell$, but dropped to 6,4 x $10^9/\ell$ at the time the animal showed clinical signs. The discontinuation of fluid therapy resulted in an uneventful recovery (Fig. 2).

Group B - Starved horse receiving fluids:

No clinical signs of discomfort were seen in this animal. The experiment had to be discontinued at the second sampling of the third period due to technical problems in producing reverse osmosis water. Fluctuations in WCC had, however, occurred throughout the three infusion periods: from 8,4 x $10^9/\ell$ at the beginning of the first infusion it rose through 8,5 x $10^9/\ell$ to 12,8 x $10^9/\ell$ at the middle and last sampling of that period. During the second period the WCC was 8,9 x $10^9/\ell$ and 10,5 x $10^9/\ell$, respectively. It was 10,3 x $10^9/\ell$ at the beginning of the third period whereafter it dropped to 7,0 x $10^9/\ell$ at the second sampling. At this point the infusion had to be discontinued (Fig. 2).

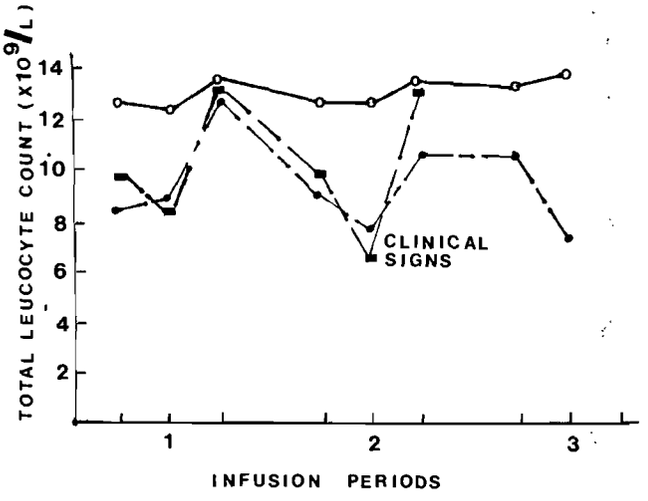

Fig. 2: ■— —■horse fed & watered ad lib. plus fluids
●——●horse starved plus fluids
○——○control horse

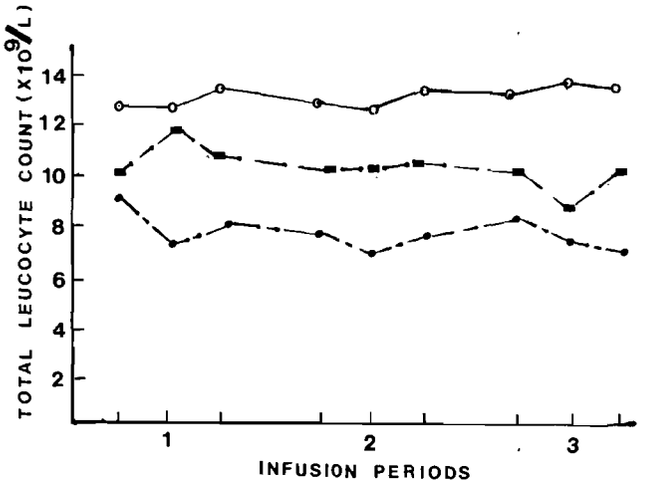

Fig. 3: ■— —■horse fed & watered ad lib.plus fluids
●——●horse starved plus fluids
○——○control horse

Group C:

Both horses receiving the commercial fluid remained clinically normal during the three infusion periods. No fluctuations in the WCC occurred (Fig. 3).

Groups A, B and C-Control horses:

The horses remained clinically normal during the duration of the experiment. No noteable fluctuations occurred in their WCC. (Fig. 1, 2 & 3).

DISCUSSION

The horse is the most sensitive domestic animal to the effects of endotoxins[3]. A sublethal dose of endotoxins causes the development of a leukopaenia as one of its first effects[10]. This endotoxin-induced leukopaenia is a very sensitive indicator of the presence of endotoxins in parenterally administered drugs[6]. Clinical signs attributable to the administration of endotoxins are: pyrexia, muscle tremors, diarrhoea and hyperpnoea[5 6 9]. These changes are constantly observed in sublethal endotoxin administration and can be used to alert one to the possible presence of endotoxins in fluids being administered.

The sources of endotoxins in this experiment were the deionised water and the water produced by reverse osmosis. The containers from which the fluids were administered probably contributed to the source of endotoxins, as they are very difficult to render endotoxin free[7]. It would appear from the more severe clinical signs seen in Group A, that the water from the deioniser probably contained more endotoxins than that produced by reverse osmosis.

The fluctuating WCC's of Group A and B during the infusions are difficult to interpret. Larger experimental groups would probably have given a clearer indication of the significance of these changes. No explanation can be given for the occasions when the WCC increased during an infusion period. The expectation was that the WCC would decrease during exposure to endotoxins with a leukocytosis developing between infusion periods[1]. A constant feature, however, was that the WCC's were at their lowest at the onset of clinical signs. The leukopaenia in endotoxic shock is thought to be due to sequestration of leukocytes in the pulmonary vasculature[3 11].

The following recommendations were made with regard to fluid therapy in the horse:

a) An attempt should be made to use commercially available fluids.

b) If the cost of such fluids is considered excessive, then:
   i) fluids should be mixed in and administered from glass containers which have been previously baked in an oven at 180°C for 4 hours to render them endotoxin free. (Product information. LAL chromogenic test. Whittaker MA Bio products 1985).
   ii) sterile and endotoxin-free water should be used
   iii) WCC determinations of animals being infused with non-commercial solutions may be an aid in confirming the presence of an endotoxaemia

ACKNOWLEDGEMENTS

The South African Defence Force is thanked for the donation of the horses and Ms A van Heerden for the leukocyte counts.

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ABSTRACT **SAMEVATTING**

ISOLATION OF *COWDRIA RUMINANTIUM* BY MEANS OF PERCOLL DENSITY GRADIENT CENTRIFUGATION AND DETECTION BY AN ENZYME-LINKED IMMUNOSORBENT ASSAY

The isolation of *Cowdria ruminantium* by means of Percoll density gradient centrifugation permits the recovery of partially purified viable populations of the organism possessing distinctly different densities. These conclusions are based upon results of analyses of density fractions by intravenous inoculation into sheep, protein determination, electron microscopy and enzyme-linked immunosorbent assay. Morphological differences were observed in the density fractions obtained from infected brain tissue and *Amblyomma hebraeum* nymphae. (Neitz, A. W. H., Viljoen, G. J., Bezuidenhout, J. D., Oberem, P.T., Putterill, J. F., Verschoor, J. A., Visser, L & Vermeulen, N. M. J., 1986. Isolation of *Cowdria ruminantium* by means of Percoll density gradient centrifugation and detection by an enzyme-linked immunosorbent assay *Onderstepoort Journal of Veterinary Research,* 53, 63-67 (1986).)

ABSTRACT **SAMEVATTING**

THE TOPOGRAPHY OF THE THORACO-ABDOMINAL VISCERA IN THE OSTRICH *(STRUTHIO CAMELUS)*

The topography of the thoraco-abdominal viscera in the ostrich was studied in 20 birds varying in age from 2 weeks to 12 months. The lungs occupied the dorsal third and the thorax, and the heart lay in the cranioventral thorax perpendicular to the long axis of the body. There was no pleural cavity. The liver was situated in the caudoventral part of the thorax, and the proventriculus occupied the left cranial part of the abdomen between the 7th vertebral rib and the acetabulum. The gizzard lay in the cranioventral part of the abdomen resting on the sternum and abdominal floor. The duodenum formed a loop from right to left, with the pancreas lying between the 2 limbs of the loop. The coiled jejunum and ileum occupied the ventral part of the abdomen between the gizzard and pelvis. The two caeca lay on either side of the terminal ileum with their apices in the pelvis. The rectum was the longest part of the intestine and could be divided into a thick proximal segment situated in the right dorsal part of the abdomen, and a thin distal part that occupied the left caudodorsal part of the abdomen. The trilobed kidneys lay along the ventral surface of the synsacrum, with the adrenal glands at their cranioventral poles. The testes lay ventrally to the cranial divisions of the kidneys, whereas the left ovary was situated ventrally to the cranial division of the left kidney. The spleen lay wedged in between the right kidney, caudal vena cava and proventriculus. The thyroid glands were situated at the cranial borders of the subclavian arteries, and the thymus lay at the base of the neck. (Bezuidenhout, A.J., 1986. The topography of the thoraco-abdominal viscera in the ostrich *(Struthio camelus). Onderstepoort Journal of Veterinary Research,* 53, 111-117 (1986).)

ABSTRACT **SAMEVATTING**

PARASITES OF DOMESTIC AND WILD ANIMALS IN SOUTH AFRICA. XX ARTHROPOD PARASITES OF THE CAPE MOUNTAIN ZEBRA *(EQUUS ZEBRA ZEBRA)*

The arthropod parasite burdens of 14 Cape mountain zebra *(Equus zebra zebra),* shot for survey purpose in the Mountain Zebra National Park in the eastern Cape Province, were determined. Three species of *Gasterophilus* larvae and 9 ixodid tick species were recovered. Larvae of *Gasterophilus percorum* were the most numerous of the fly larvae recovered and *Margaropus winthemi* was the most abundant tick.

Two horses examined in the park were infested with 3 species of *Gasterophilus* larvae and 7 species of ixodid ticks. (Horak, I.G., Knight, M.M. & De Vos, V., 1986. Parasites of domestic and wild animals in South Africa. XX. Arthropod parasites of the Cape mountain zebra (Equus zebra zebra). *Onderstepoort Journal of Veterinary Research,* 53, 127-132 (1986).)

RESEARCH NOTE **NAVORSINGSNOTA**

ARTIFICIAL INSEMINATION OF ANGORA- AND BOERGOATS WITH DEEP-FROZEN SEMEN

R. LAWRENZ*

ABSTRACT: Lawrenz R. **Artificial insemination of Angora- and Boergoats with deep-frozen semen.** *Journal of the South African Veterinary Association* (1986) 57 No. 2, 109-111 (En) P.O. Box 1539, 9500 Kroonstad, Republic of South Africa.

A total of 218 goats were inseminated with thawed frozen semen and a non-surgical intra-uterine insemination technique. Of the various groups inseminated the percentage Boergoat does kidding was 60,8% (46) and 70,9% (31), the percentage Angoragoat does kidding was 62,3% (53), 61,8% (68) and 60,0% (20). A dose of 75 x 10^6 sperm /ml was found to be suitable for intra-uterine insemination of Angora does.

Key words: Frozen Angora- and Boergoat semen, intra-uterine insemination.

INTRODUCTION

The use of frozen goat semen in planned breeding programmes is mainly practised, although to a limited extent, in European countries like France, Switzerland, Norway and Czekoslovakia[6]. Various authors have, however, reported on the successful artificial insemination with frozen goat semen[1,3,5,9]. Whilst most experimental work on this subject is being done on dairy goat breeds, limited information is available on the fertility of frozen Angora goat semen[2,7,9,12].

Successful freezing of Boergoat semen has been described by Rossouw[10] during 1974, but no results of artificial insemination with this semen are available. Although satisfactory conception rates are achieved with artificial insemination with frozen semen, results often vary quite considerably between different authors: a kidding percentage of 65,1% has been reported by Corteel et al.[3] following double insemination of 1093 dairy goats with frozen semen containing 150 – 200 x 10^6 spermatozoa, whereas V.d. Westhuizen et al.[12] reported a conception rate of 27% and 50% in Angora does inseminated with 0,25 ml and 0,5 ml frozen semen, respectively. In a comparative study Loubser et al.[7] found that only 34,8% of does inseminated with frozen semen kidded compared to 90% of does inseminated with fresh semen.

In a series of experiments comparing the fertility of fresh and frozen Angora goat semen Ritar & Salamon[9] found an improvement in fertility with increasing depth of insemination into the genital tract – the effect being more pronounced with frozen thawed than fresh diluted semen. With intra-uterine deposition of the thawed semen, kidding rates of 67,4% (48 does) and 83,3% (18 does) were obtained. The purpose of the present study was to determine if Angora- or Boergoat semen could be used successfully in a planned breeding programme.

MATERIAL AND METHODS

During August 1983 semen was collected from a mature Boergoat ram and during March 1985 from a mature Angora goat ram with the use of an artificial vagina. A double ejaculate of each ram was collected twice weekly. For the purpose of processing and freezing of the semen, the method as described by Summermatter (P. Summermatter 1983 AI Station Bütschwil, CH-960 Bütschwil, personal communication) was used. With the use of the improved Neubauer cell counting chamber, the number of spermatozoa per mm^3 in the undiluted ejaculates was counted. After addition of the calculated volume of diluent, semen was filled into the plastic straws, each containing a 0,2 ml aliquot of diluted semen. In the case of the Boergoat ram, each dose of semen contained 200 x 10^6 spermatozoa, while in the Angora goat straws containing two different sperm concentrations namely 150 x 10^6 and 75 x 10^6 spermatozoa, were prepared. The semen was subsequently stored in liquid nitrogen and thawed at 70°C for 10 seconds just prior to insemination.

Goats on 3 different farms were inseminated. All animals were kept under extensive grazing conditions, except in the case of the Angora goats, which had additional access to green lucerne for one hour per day.

A non-surgical intra-uterine insemination technique as described by Fougner[5] was used. Inseminations were only carried out in does where intra-uterine semen deposition was possible. Vasectomised teaser rams were used to identify does in oestrus, which were then inseminated approximately 12 hours after the onset of standing heat.

During November 1984 (outside the normal breeding season) a total of 46 Boergoat does were inseminated (Experiment I) whilst a total of 31 Boergoat does were inseminated during April 1985 during the normal breeding season (Experiment II). The Boergoat semen used had been stored in liquid nitrogen for 15 and 20 months, respectively.

In Experiments III and IV (April 1985) a total of 180 Angora does were treated with medroxyprogesterone-acetate intravaginal sponges (Repromap, Upjohn) for 12 days and on the day of sponge removal, 300 I.U. Pregnant Mare Serum (Fostim, Upjohn) was injected intramusculary. Does were inseminated during the second oestrus starting approximately 19 days after sponge withdrawal. Between the 19th and 23rd day after sponge removal a total of 121 does were inseminated: 53 with semen containing 150 x 10^6 sperm per dose (Experi-

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Table 1. Results of artificial insemination with frozen semen.

Experiment	No of does inseminated	65 day Nr-rate	65 day Nr-R%	No of does kidded	% does kidded	No of kids per doe
I	46	31	67,4	28	60,8	1,64
II	31	24	77,4	22	70,9	1,90
III	53	35	66,0	33	62,3	1,15
IV	68	44	64,7	42	61,8	1,35
V	20	13	65,0	12	60,0	1,58

Nr = non-return
R = return

ment III) and 68 with semen containing 75 x 10^6 sperm per dose (Experiment IV).

A seperate group of 20 Angora does were synchronised with medroxyprogesterone acetate intravaginal sponges (Repromap, Upjohn) which were withdrawn on the 11th day. Ten mg of dinoprost (Lutalyse, Upjohn) and 400 I.U. Pregnant Mare Serum (Fostim, Upjohn) was injected intramuscularly 48 hours before sponge removal[3]. A double insemination was carried out with semen containing 75 x 10^6 sperm per dose, 31 and 48 hours after removal of sponges (Experiment V).

For a period of 65 days after the does were inseminated, vasectomised teaser rams were allowed amongst the does for one hour in the morning and evening. Does returning to oestrus were taken to a fertile ram for handmating – their ear numbers recorded – and then put into a new group. The does not returning to oestrus within 65 days after insemination were left in one group until kidding. The actual number of does kidding as a percentage of the total number of does originally inseminated was taken as the actual percentage of does kidding. The number of kids per doe was also recorded to work out the average number of kids per doe.

RESULTS

The results of the present study are summarised in Table 1. It is quite clear from these results, that a higher kidding percentage as well as more kids per doe were obtained when Boergoat does were inseminated during the normal breeding season (Experiments I and II).

It is also quite clear, that no marked difference occurred in the two groups of Angora does inseminated with semen containing different sperm numbers (Experiment III and IV). In Experiment V, where synchronised Angora does were inseminated at fixed times, more kids per does were born, when compared with insemination at natural oestrus (Experiments III and IV).

DISCUSSION

The results obtained in Experiments I – IV show that satisfactory conception rates can be achieved with a single intra-uterine insemination. These results are in accordance with those of Fougner[5], who also, after single intra-uterine insemination, obtained a kidding percentage of above 60%.

From Experiments III and IV it can be concluded, that a single dose of frozen goat semen containing 75 x 10^6 spermatozoa per dose is sufficient to obtain satisfactory conception rates. A definite advantage of using semen containing only 75 x 10^6 spermatozoa per insemination dose, is that one ejaculate can be used much more efficient i.e. at least double the number of insemination doses can be prepared from a single ejaculate compared to doses containing a concentration of 150 – 200 x 10^6 spermatozoa.

The lower concentration, however, neccesitates intrauterine semen deposition, which is much more time consuming, and the use of special insemination equipment is required.

Corteel & Paquinon[4] are of the opinion that passage through the cervical canal, when frozen semen is used for insemination, is not neccessarily the relevant key to success to obtain satisfactory conception rates. This view is supported by the results of experiments carried out by Corteel et al.[3], whereby a 65,1% kidding rate was achieved following insemination in the os cervix with frozen sperm containing 200 x 10^6 spermatozoa.

As shown in Experiments I – V, there is a difference between the 65 day non-return rate and the actual number of does kidding: in these experiments up to a 10% difference was observed. In goats this difference has also been described by Fougner[5], and Menger et al.[8], who found a 25% difference in the number of sheep not returning to oestrus and the number of sheep lambing. It can therefore be concluded, that does not returning to oestrus after insemination cannot always be assumed to be pregnant.

The success of an insemination programme for goats in South Africa, whereby frozen semen is to be used, will be determined mainly by factors such as the skills and ability of technical personnel to successfully freeze semen, correct insemination technique, satisfactory conception rates, the interest of the official breeding organisations and farmers with good flock management and interest in such a programme.

Due to the relative high cost of this procedure, such a programme would probably only be economically justifiable in certain stud animals, where planned matings should take place between performance tested rams and selected does.

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ABSTRACT

SAMEVATTING

HEARTWATER: AN *IN VITRO* STUDY OF THE ULTRASTRUCTURE OF *COWDRIA RUMINANTIUM*

Notwithstanding morphological differences, the ultrastructure of *Cowdria ruminantium* cultured *in vitro* concurred to a large extent with that in previous *in vivo* studies. Two distinct forms of the organism, elementary and reticulate bodies, and a 3rd group of intermediate organisms were identified. Organisms within a particular vacuole were generally a specific form, but in cells containing many colonies different forms were present in the same colony. Most organisms were enveloped by 2 membranes and a few were surrounded by a 'capsule'. *C. ruminantium* multiplies mainly by binary fission, but it appears that multiplication can also take place by means of budding. The taxonomy of *C. ruminantium* is briefly discussed. (Prozesky, L., Bezuidenhout, J. D. & Paterson, Camilla L., 1986. Heartwater: An *in vitro* study of the ultrastructure of *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research,* 53, 153-159 (1986).)

ABSTRACT

SAMEVATTING

THE HELMINTH PARASITES OF VARIOUS ARTIODACTYLIDS FROM SOME SOUTH AFRICAN NATURE RESERVES

The helminth species composition and helminth burdens of 4 grey duikers, 12 bushbuck, 2 nyala, 2 giraffe, a steenbok, an oribi, a waterbuck and a tsessebe from the Kruger National Park (KNP); of a steenbok and a greater kudu from the farm Riekerts Laager, Transvaal; of a single blue duiker from the Tsitsikama Forest National Park, and of a blue wildebeest, a red hartebeest, a gemsbok and 2 springbok from the Kalahari Gemsbok National Park (KGNP) were collected, counted and identified.

New parasite records are: *Agriostomum equidentatum* from the gemsbok, *Cooperia neitzi* from the bushbuck, *Cooperia* sp. from the gemsbok and the red hartebeest, *Cooperia yoshidai* from the waterbuck and the tsessebe, *Dictyocaulus viviparus* from the bushbuck, *Haemonchus bedfordi* from the waterbuck, *Haemonchus contortus* from the gemsbok, *Haemonchus krugeri* from the steenbok from the KNP, *Impalaia nudicollis* from the gemsbok and the red hartebeest, *Impalaia tuberculata* from the oribi and the waterbuck, *Impalaia* spp. from the kudu, *Longistrongylus meyeri* from the steenbok from Riekerts Laager and the gemsbok, *Longistrongylus sabie* from the steenbok from the KNP, *Longistrongylus schrenki* from the tsessebe, *Parabronema* sp. from the tsessebe and the red hartebeest, *Paracooperia serrata* from the gemsbok and the steenbok from the KGNP, *Pneumostrongylus calcaratus* from the bushbuck, *Strongyloides* sp. from the gemsbok, *Trichostrongylus* sp. from the gemsbok, the red hartebeest and the steenbok from the KGNP, *Trichostrongylus axei* from the blue duiker, *Trichostrongylus falculatus* from the bushbuck and the oribi, *Trichostrongylus instabilis* from the bushbuck, the steenbok from the KNP and the oribi and *Trichostrongylus thomasi* from the grey duikers and tsessebe.

Host specificity of the parasites was not marked and crossinfestation was common. This was not true for the giraffe, since none of the helminths of these animals were found in the antelope and vice versa. (Boomker, J., Horak, I.G. and De Vos, V., 1986. The helminth parasites of various artiodactylids from some South African nature reserves. *Onderstepoort Journal of Veterinary Research* 53, 93-102 (1986).)

ABSTRACT

SAMEVATTING

FOOT-AND-MOUTH DISEASE AND THE AFRICAN BUFFALO *(SYNCERUS CAFFER)*. 1. CARRIERS AS A SOURCE OF INFECTION FOR CATTLE

Ten pregnant buffalo cows, six of which were subsequently shown to be carriers of SAT 1, 2 and 3 viruses, were captured in the Kruger National Park (KNP) and allowed to calve in captivity. The buffalo cows and calves were seperated by a fence from 6 FMD susceptible cattle but the buffalo and cattle were obliged to use common drinking troughs and hay racks. Over a period of 15 months, during which the buffalo calves lost their maternally-derived immunity, neither the buffalo calves nor the susceptible cattle became infected with FMD virus. By the end of the observation period, however, only 1 buffalo cow still had detectable virus in its oesophageal/pharyngeal specimens. (Bengis, R. G., Thomson, R, S., De Vos, V & Pini, A., 1986. Foot-and-mouth disease and the African buffalo *(Syncerus caffer)*. 1. Carriers as a source of infection. *Onderstepoort Journal of Veterinary Research,* 53, 69-73 (1986).)

CASE REPORT **GEVALVERSLAG**

VERDAGTE OPIUMVERGIFTIGING IN TWEE JONG HONDE

J S J ODENDAAL*

ABSTRACT: Odendaal J.S.J. **Suspected opium poisoning in two young dogs.** *Journal of the South African Veterinary Association* (1986) **57** No. 2, 113-114 (Afrik). Department of Zootechnology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

Two young dogs which played in a domestic garden in which the opium poppy (*Papaver somniferum*) grew as wild flowers, developed euphoric as well as other nervous signs, reminiscent of opium poisoning. No medicinal treatment was given. The one dog recovered in one day while the other one took one to two weeks to recover.
Key word: Dogs, suspected opium poisoning, *Papaver somniferum*.

INLEIDING

Dit is algemeen bekend dat sekere *Papaver spp* opium en opiumderivate bevat. Hierdie stowwe kom veral in hoë konsentrasies voor in die melkagtige vloeistof wat in die onryp sade in die lente vorm. Ru-opium bevat ongeveer vier-en-twintig alkaloïede waarvan morfien, kodeïn en papaverien die bekendste is. Opium word al vir eeue in die Ooste as pynstiller gebruik weens sy effek op die sentrale senuweestelsel. Dit word egter ook aangewend in diarreemiddels, krampweerders en hoesonderdrukkers. In die Weste word opium veral as verslawingsmiddel gebruik.

Morfien in honde toon 'n kort voorafgaande periode van stimulasie van die sentrale senuweestelsel, wat gekenmerk word deur rusteloosheid, kortasemigheid, salivasie, naarheid, braking, urinering, en ontlasting. Hierdie kliniese tekens verminder geleidelik en word gevolg deur domheid (stupor) wat dui op onderdrukking van die serebrale korteks[1].

Die respirasie sentrum, wat ook aanvanklik gestimuleer word, dra by tot die kortasemigheid en 'n meegaande tydelike verhoging van liggaamstemperatuur. As onderdrukking eers plaasvind, kan die liggaamstemperatuur daal as gevolg van 'n meegaande daling in bloeddruk. Die respirasiesentrum wat ook onderdruk word, sal in normale, gesonde honde wat klein dosisse toegedien is, nie die suurstof verbruik met meer as 10% verminder nie[1].

Alhoewel morfien die parasimpatiese toevoer na die iris aktiveer, word die miotiese effekte ge-antagoniseer deur verhoogde katekolamien afskeiding vanaf die adrenale, en dit veroorsaak midriase[1]. Honde toon dus gewoonlik midriase, maar kan ook miose toon.

Die alkaloïede word in die maag swak geabsorbeer weens die maag se lae pH. In teenstelling met die maag, word alkaloïede goed vanuit die alkaliese dunderm geabsorbeer. Opium word parenteraal vinnig geabsorbeer en is effektief binne 10 tot 15 minute. Morfiene word in honde vinnig uitgeskei; 65% van die totale dosis word in die uriene binne 24 uur uitgeskei, en sowat 20% die volgende vier dae[2]. Indien die effek van morfien verleng word, kan dit die urienuitskeiding verminder tot 10% of minder as die normale uitskeiding, as gevolg van die vrylating van anti-diuretiese hormoon[1].

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Die toksiese dosis varieer grootliks tussen spesies en individue. Verskeie ander faktore kan ook die toksisiteit beïnvloed. So is daar 'n moontlike verwantskap tussen die vatbaarheid in opiumvergiftiging en die graad van ontwikkeling van die sentrale senuweestelsel. Jonger diere sou dus hiervolgens minder vatbaar wees as ouer diere, maar tog is bevind dat die omgekeerde waar is[2]. Dodelike dosisse in die hond wissel van 110 – 220 mg/kg met subkutane of intraveneuse inspuitings. Met hoë dosisse morfien word konvulsies soortgelyk aan strignien, in die meeste spesies gestimuleer[1].

GESKIEDENIS, KLINIESE ONDERSOEK EN DIAGNOSE

'n Klein (5kg) Toy Pom-kruis reuntjie (7 maande oud), is ingebring met die klagte dat hy skielik eienaardige gedrag begin toon het. Die hondjie was lêerig, maar was terselfdertyd rusteloos. Skuim en kwyl was om die hond se bek en hy het aanvanklik naar voorgekom. Geen vomisie is egter gesien nie. Die kliniese tekens het skielik ontstaan en daar is aanvanklik vermoed dat die hondjie allergies was vir iets waaraan hy gekou het.

Weens die eienaardige tekens is die hondjie vir meer as 'n uur onder observasie gehou. Die fisiese ondersoek het getoon dat die pasiënt mioties was. Die volgende gedragsimptome was waargeneem: Die hondjie het verkies om vir kort periodes te lê eerder as om te staan of loop; as hy geloop het, het een of meer bene onder hom geswik en sy beweging was ataksies. Dit het voorgekom asof hy nie bewus was van die kliniese ondersoek nie, terwyl hy andersins 'n hondjie met aktiewe gedrag was. Sy oë het gestaar en die diertjie het hipnoties voorgekom. Al reaksie wat die pasiënt getoon het as 'n mens sy aandag probeer trek, was om sy stert te waai. Terwyl die hondjie nog onder observasie was, is die eienaar se ander hondjie ingebring met soortgelyke kliniese tekens. Hierdie tweede geval was die eerste hondjie se suster van dieselfde werpsel en met 'n massa van 4 kg. Haar kliniese tekens was minder uitgesproke en daar was midriase. By verdere ondersoek het dit geblyk dat die papawerplante in die tuin deur die honde plat gespeel is en dat daar jong sade (dit was in die lente) gevind is wat stukkend gekou is.

Die plante was geïdentifiseer as die opium-papawer (*Papaver somniferum*). 'n Diagnose van verdagte opiumvergiftiging was gemaak na aanleiding van die geskiedenis en gedrag.

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BEHANDELING EN RESULTATE

Geen medikasie was vir die opiumvergiftiging toegedien nie. Daar was aanbeveel dat die hondjies tyd gegun word om die gifstof self uit te skei. Die hondjies is die volgende dag weer ondersoek en die tefie wat die mindere simptome getoon het, was feitlik weer normaal. Die reuntjie was effe beter in die sin dat hy meer belang gestel het in sy omgewing en meer gekoördineerd geloop het. Hy was steeds nog lêerig en was steeds mioties. Albei hondjies het geëet. Volgens die eienaar het die tefie volkome herstel twee dae na die aanvang van die kliniese tekens, terwyl die reuntjie ongeveer 'n week geneem het om van sy lêerige gedrag ontslae te raak. Ná herstel was die reuntjie egter merkbaar meer humeurig as normaal. Hy was veral kwaai met alle vreemde mense wat die erf betree het. Hy was egter nie aggressief teen sy eienaar nie. Alhoewel die reuntjie en die tefie groot speelmaats was, wou die reuntjie glad nie met haar speel nie. Volledige herstel na normale gedrag, soos vóór die vergiftiging, het ongeveer twee weke geduur.

BESPREKING

Selfvergiftiging deur honde met 'n opiumplant is 'n raar verskynsel. Moontlike omstandighede is wanneer jong hondjies wat aan alles kou, in kontak kom met papawerplante wat hulle jong sade in die lente vorm. Dit is dan ook wat in hierdie geval gebeur het.

As die verdagte diagnose as korrek aanvaar word, kan die feit dat die een hondjie erger kliniese tekens as die ander een getoon het, aan drie moontlikhede toegeskryf word. Eerstens kon die tefie 'n kleiner dosis per massa ingeneem het en tweedens kon daar 'n individuele verskil in vatbaarheid vir die gif tussen die twee hondjies bestaan het. Derdens, alhoewel braking nie deur die eienaar waargeneem is nie, kon die tefie dalk van die sade uitgebraak het.

Die dosis was vir altwee hondjies in elk geval nie hoog genoeg om onomkeerbaar te wees nie. Die kliniese tekens is nie as ernstig genoeg beskou om spesifieke behandeling toe te pas nie en omdat die herstel volkome was, is hierdie besluit as korrek bewys. 'n Interessante bevinding was die postdepressiewe aggressie wat die reuntjie getoon het. Om herhaling van hierdie toevallige verdagte vergiftiging te voorkom, is die papawers in die tuin uitgeroei.

ERKENNING

Dank word betuig aan Prof H J T Venter van die Departement Plantkunde, Universiteit van die Oranje Vrystaat, Bloemfontein, vir die identifikasie van die plant.

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CASE REPORT **GEVALVERSLAG**

BACTERIAL MYOCARDITIS SECONDARY TO PARVOVIRUS ENTERITIS IN A PUPPY

I.B.J. VAN RENSBURG* and R. MEINTJES**

ABSTRACT: Van Rensburg I.B.J.; Meintjes R. **Bacterial myocarditis secondary to parvovirus enteritis in a puppy.** *Journal of the South African Veterinary Association* (1986) **57** No. 2, 115-116 (En). Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

A purulent bacterial myocarditis, secondary to parvovirus enteritis was diagnosed in a 3-month-old St Bernard puppy. The clinical course was of 2 days duration and was characterized by pyrexia, severe vomition, haemorrhagic diarrhoea and dehydration. Post mortem examination revealed a haemorrhagic enteritis and multifocal purulent myocarditis. Histopathological examination proved that the latter was of bacterial origin. It is postulated that this resulted from a bacteraemia secondary to the intestinal lesions caused by parvovirus infection.

Key words: Bacterial myocarditis, canine parvovirus enteritis, bacteraemia.

INTRODUCTION

Bacteraemia as a complication of parvovirus enteritis was manifested by a multifocal purulent myocarditis in a puppy. This implies an increased permeability to micro-organisms of the damaged intestinal mucosa. As this has bearing on the treatment regime of canine parvovirus (CPV) patients, this case is reported.

CASE HISTORY

A 3-month-old St Bernard bitch with a body mass of 5 kg was admitted to the Outpatients Clinic at the Faculty of Veterinary Science, University of Pretoria with a history of vomition and diarrhoea which had a sudden onset. The puppy had not been vaccinated against CPV disease. Clinically it had a rectal temperature of 38°C and was in a poor condition. The stool was watery and haemorrhagic. Faecal flotation was positive for *Ancylostoma* eggs while the haemagglutination (HA)-test on a stool sample was strongly positive for CPV, yielding a titre of 1:1024. Treatment consisted of disophenol (Ancylol, SA Cyanamid), amoxycillin trihydrate (Clamoxyl, Beecham) given parenterally and electrolytes (Electrosol, Milvet) *per os* in small quantities at short intervals. Twenty four hours later the dog was very dehydrated and was admitted to the Department of Medicine where an intravenous drip (Plasmalyte B, Sabax), to which 10 ml of a 15% KCl solution and 100 ml of 50% dextrose was added per litre of Plasmalyte, was administered. The systemic antibiotic therapy was repeated. Despite continued treatment the condition of the patient deteriorated gradually, it became anorectic and depressed and died about 36 hours after admission. The cadaver was refrigerated at 4°C for ± 10 hours before a post mortem examination was carried out.

Macroscopical findings

The peri-anal area was soiled with faeces containing fresh blood. There was a distinct anaemia, congestion and oedema of the superficial lymph nodes, congestion of the lungs and oedema of the thymus. A mild hydropericardium, fatty change of the liver, nephrosis and congestive splenomegaly were also recorded. The myocardium of especially the left ventricular wall and septum revealed focal disseminated pale yellow areas which varied from pinpoint to *c* 1 mm in diameter.

The intestinal lesions consisted of a catarrhal duodenitis and jejunitis and a severe haemorrhagic ileitis. The mucosa had a flattened appearance with a distinct absence of discernable villi. The large intestine was filled with a haemorrhagic watery content, but the mucosa was of normal appearance.

Specimens of the intestine, myocardium, lymph nodes and spleen were fixed in 10% buffered formalin for histopathological examination. No specimens were submitted for bacterial isolation.

Microscopical findings

The intestinal lesions were typical of those of CPV-enteritis[4,6,7]. The major findings were denudation of villi and loss of epithelium from the crypts resulting in a collapse of the mucosa. The few remaining epithelial cells in some of the crypts were attenuated and flattened, their nuclei contained large nucleoli, but distinct intranuclear inclusions were not noticed. Some crypts were dilated and filled with cellular debris. The lymphoid tissues in the lymph nodes and spleen manifested suppressed activity.

The myocardium revealed numerous foci of an acute, embolic bacterial, myocarditis characterised by necrosis and rarefaction of the myocardial fibres in the immediate vicinity of homogenous populations of fairly large, Gram-positive bacilli. Moderate numbers of neutrophils and macrophages were present in most of the foci but a few bacterial colonies were noticed in the absence of an inflammatory response.

DISCUSSION

Bacteraemia concomitant with parvovirus infection in a pup was recently reported by Kreeger et al.[3]. The organisms involved in this instance were normal inhabitants of the intestine. Kahn[2] showed that in cats with feline panleukopaenia, intestinal bacteria can gain access to the systemic circulation while Reggiardo & Kaeberle[8] demonstrated the occurrence of a similar

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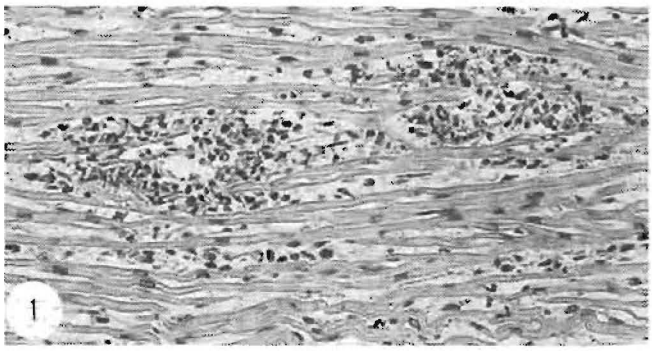

Fig. 1: Foci of bacterial myocarditis. HE X100.

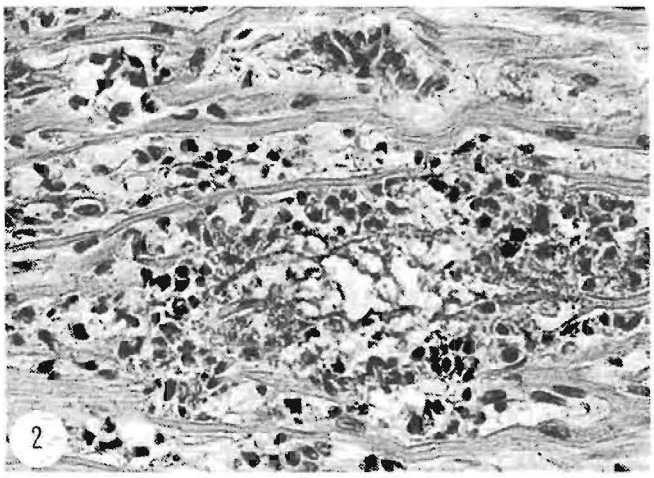

Fig. 2: As above. Bacteria, macrophages and some neutrophils visible. HE X 400.

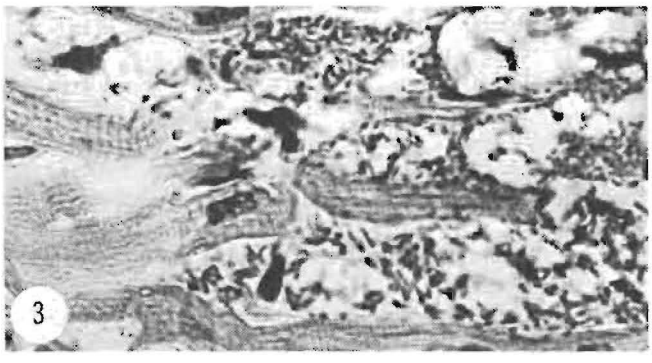

Fig. 3: Bacteria, necrosis and lysis of myocardial fibres. HE X 1000.

situation in cattle inoculated with bovine viral diarrhoea virus.

In a study with specific pathogen free cats, Carlson et al.[1] demonstrated a much less serious effect than in cenventional cats when infected with feline panleukopaenia virus, thus suggesting that the intestinal flora plays a major role in the development of diarrhoea and shock in affected cats (and dogs?).

It seems likely that at least 2 factors may predispose to bacteraemia associated with CPV infection. The severe damage to the intestinal mucosa by the virus which is likely to increase the permeability of the mucosa is thought to play a major role. Certain cases of CPV enteritis show unusual numbers of bacteria in the intestinal serosa and mesentery (Van Rensburg I.B.J. – unpublished data). Maccartney et al.[5] postulated that the large quantity of antibodies observed in the intestinal contents with CPV enteritis is the result of extensive mucosal damage and direct leakage of humoral antibodies into the lumen. This is also indicative of increased permeability. The second factor is the leukopaenia (absolute neutropaenia and in many cases absolute lymphopaenia) characteristic of CPV infection[4,5] which leads to a compromised immunological defence. It is interesting to note that despite the lympholytic action of CPV, there is no rapid, good humoral antibody response in patients suffering from this infection[5].

The predispostion to bacteraemia is of great significance in the treatment of patients affected by CPV where the systemic use of broad-spectrum antibiotics is strongly indicated for several days together with the symptomatic treatment.

In the case described in this paper, bacterial isolation was infortunately not done. The absence of an inflammatory response around a few of the bacterial colonies suggests that the bacteraemia was an agonal phenomenon. It is difficult to estimate the age of the lesions where overwhelming numbers of bacteria are associated with marked necrosis and degeneration of the myocardium in the presence of moderate numbers of neutrophils and macrophages. The most feasible explanation is that bacterial multiplication continued in the myocardium for some time after death despite the fact that the cadaver was refrigerated.

The findings reported in this paper as well as those reported by Kreeger et al.[3] warrants further investigation to establish the incidence of bacteraemia as a complication of CPV infection.

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CASE REPORT **GEVALVERSLAG**

THE PATHOLOGY OF A CASE OF BILIARY ATRESIA IN A FOAL

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ABSTRACT: Bastianello Stella S.; Nesbit J.W. **The pathology of a case of biliary atresia in a foal.** *Journal of the South African Veterinary Association* (1986) **57** No. 2, 117-123 (En) Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

The pathological features of biliary atresia in a foal are described. A 4-week-old American Saddler foal was presented for autopsy following an illness characterised by clinical features indicative of hepatic failure. The significant macroscopical lesions occurred in the liver which was extremely enlarged, mottled in appearance and indurated. Bile stasis was evident. Lobular distinction was absent and on sectioning, large bile ducts were absent. A moderate ascites, hydrothorax and hydropericardium and a mild anasarca and intermuscular oedema accompanied the hepatic lesion. The diagnosis of biliary atresia was determined by the histopathological features of bile duct proliferation and extensive replacement fibrosis. The condition is compared to extrahepatic and intrahepatic biliary atresia of man and evidence is presented for regarding this case to be one of extrahepatic origin.

Key words: Pathology, biliary atresia, equine.

INTRODUCTION

Congenital biliary atresia is a well documented although rare entity of human infants, in which it may be either intrahepatic or extrahepatic in origin[3]. In animals, biliary atresia is extremely rare but has been reported in rhesus monkeys, mice, pigs and a foal[2,7].

The aetiology of biliary atresia in equines is completely unknown and there is only one record of the condition in foals[7]. This report describes the pathology of a case of biliary atresia in a foal.

HISTORY AND CLINICAL SIGNS

The foal presented for post-mortem examination was the fourth of an American Saddler mare. Although the foal was born 2 weeks prematurely, the gestation and birth of the foal had been uneventful. As far as could be ascertained, the 3 previous foals are still in good health. This particular foal progressed well until 3 weeks of age when it became listless, anorectic and icteric. A few days later, it developed a recurring high fever, polydipsia, polyuria and resultant dehydration. The animal was treated intensively with antibiotics, multivitamin preparations, liver stimulants and fluid therapy. The foal did not show any improvement and died within a week of the onset of illness when it was presented for autopsy.

PATHOLOGY

A complete post-mortem examination was performed. Specimens of the liver and a series of other tissue were collected in formalin for routine histopathological examination. Routine sections were prepared from paraffin-embedded tissue blocks and stained with haematoxylin and eosin (HE). Selected sections of the liver were stained with the Hall's stain for biliverdin, Berlin blue for haemosiderin, Masson's trichrome for collagen, the Gomori reticulin impregnation (GRI) method for reticulin fibres, periodic-acid-Schiff (PAS) for mucopolysaccharides, and PAS, Schmorl's and the long Ziehl-Neelson (ZN) methods for lipofuscin[4].

Macroscopic Pathology

The carcass was in a fairly poor condition. A moderate icterus was present. Oedema was evident as a mild anasarca and intermuscular oedema involving the large muscles of the back and limbs together with a moderate ascites, hydrothorax and hydropericardium.

The liver was extremely enlarged and firmer than normal. The capsular vessels were distended and tortuous and numerous subcapsular petechiae and ecchymoses were evident (Fig. 1). The colour varied from pale pink to yellowish-white interspersed with blotchy purple areas. The lobular architecture was no longer discernible. When sectioned, no large bile ducts were evident. There was no obvious abnormality of the extrahepatic bile duct, but the patency thereof was unfortunately not determined. There was an irregular deposition of fibrous tissue which occurred as strands, a few mm to several cm in width, both on the surface and within the substance of the liver (Fig. 2). The fibrous tissue occasioned the variation in colour described above. After formalin fixation, the hepatic tissue appeared as islands of dull green tissue interspersed amongst the fibrous strands.

Other findings included moderate congestion and oedema with multifocal petechiae and ecchymoses of the lungs; moderate lymphiod atrophy of the spleen and lymph nodes; moderate congestion of the entire gastrointestinal tract; absence of gastric contents; the presence of pasty, grey colonic contents and a mild serous atrophy of the mesentric fat depots.

Histopathology

The predominant finding was a pronounced proliferation of bile ducts which were surrounded by a variable amount of fibrous tissue (Fig. 4). The hepatic tissue was distinguishable as islands of hepatocytes interspersed amongst this mass of ductular and fibrous tissue. In the less severely affected areas the hepatic tissue exhibited a degree of confluence so as to form the predominant component of the tissue (Fig. 3).

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The newly formed or actively proliferating bile ducts encompassed lumens of varying size and shape. The lining cells were cuboidal with an amphophilic cytoplasm and round to oval, vesicular nuclei. Occasional duplication of the lining epithelium was evident (Fig. 5) as well as a relatively high mitotic index averaging 2 mitoses per 40 x magnification field. In many instances the lining cells had separated from the underlying basal lamina (Fig. 5). Several of these cells exhibited vacuolar degeneration, many of the vacuoles containing homogenous acidophilic inclusions of varying size (Fig. 6). Focal flattening of the lining epithelium was evident in many of the more mature bile ducts. The lumens of these ducts were mostly empty although some contained cytoplasmic debris or the occasional, isolated desquamated and necrotic epithelial cell (Fig. 5). A noteworthy feature was the complete absence of bile within the lumens of the proliferating bile ducts.

The fibrous tissue component consisted mostly of fibroblastic or mature collagenous tissue (Fig. 3-5). The fibroblastic tissue consisted mostly of mature, elongated, fibroblasts, fewer plump, immature fibroblasts, moderate amounts of reticulin fibres and some collagen fibres. Many of the mature fibroblasts exhibited pyknosis or karyorrhexis whilst some of the immature fibroblasts were in the process of mitosis. The collagenous tissue was composed of collagen fibres which varied form strands a few fibres thick to wide bands, the latter occurring especially in the vicinity of blood vessels. There was moderate oedema of the fibrous tissue and mineralisation of some of the reticulin fibres.

The islands of hepatic tissue exhibited loss of architectural structure. In the less severely affected portions, portal triads or central veins were occasionally discernible. The portal triads were noteworthy because of 2 characteristics; firstly, a total absence of normal bile ducts and, secondly, vascular hypertrophy or hyalinisation. Another significant feature was parenchymal cholestasis characterised by distension of the canaliculi with bile. The hepatocytes revealed prominent nuclear and cytoplasmic changes. These included cytoplasmic degeneration (Fig, 7 & 8) and pigmentation, anisonucleosis and a moderate degree of binucleation (Fig. 7) and multinucleation (Fig. 8). The hepatocytes in the smaller islands of hepatic tissue, almost without exception, showed varying degrees of degeneration from cloudy swelling through hydropic changes to hyaline droplet degeneration (Fig. 7 & 8). On the other hand, only a small proportion of the hepatocytes within the larger islands exhibited these degenerative changes. Many of the degenerated hepatocytes contained varying amounts of lipofuscin and bile pigments. Similar pigments were also present within the Kupffer cells which appeared prominent and hyperplastic.

Focal scattered round cell aggregates were present at the edges of the remaining hepatic tissue. These aggregates were composed predominantly of small and large lymphocytes, some macrophages and isolated neutrophils and plasma cells. Many of these cells exhibited pyknosis or karyorrhexis.

DISCUSSION

Van der Luer & Kroneman[7] reported on the passage of soft, grey clay-like faeces and clinical pathological tests indicative of total obstruction of bile flow in a foal with extrahepatic biliary atresia. Pasty grey contents were present in the colon of this foal but no clinical pathological tests were performed. The jejunal intussusception was regarded as a terminal and incidental event not connected to the hepatic pathology.

The diagnosis of biliary atresia in this foal was based on the histopathological features of the liver. These comprised a diffuse and extensive bile duct proliferation and hepatocellular depletion with fibrous replacement resulting in eventual effacement of the lobular architecture. An important diagnostic feature was the complete absence of any pre-existing bile ducts in the remaining portal triads. The bile duct proliferation was regarded as an abortive attempt to engender continuity of the biliary excretory pathway in the affected neonatal animal. The failure to form a functional biliary tree led to an ongoing, progressive proliferation of bile ducts in a disorganised fashion resulting in parenchymal degeneration and atrophy, replacement fibrosis and consequent loss of architectural structure. The hyalinisation of the portal vessels together with the

Fig. 1: Grossly enlarged pale liver with subcapsular haemorrhages.

Fig. 2: Cross-section of the liver in a less severely affected area. The white areas represent the fibrous tissue traversing the dark coloured hepatic tissue.

Fig. 3: Severe perilobular fibrosis and bile duct proliferation. Note absence of portal triads within the hepatic tissue. HE X 40

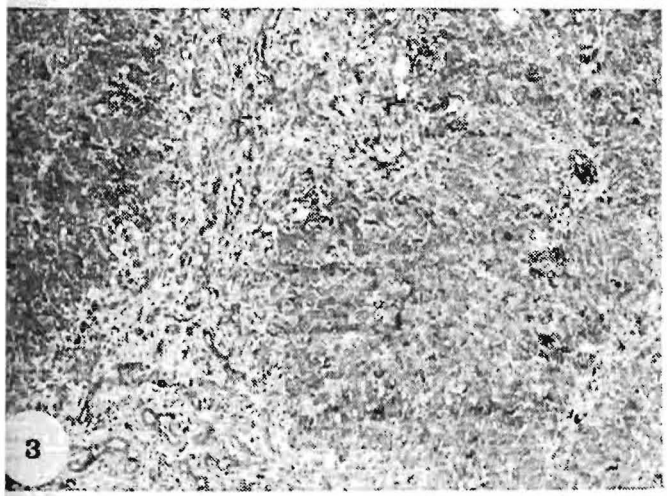
Fig. 4: Marked bile duct proliferation. Note the extensive fibrosis and parenchymatous remnants (P). HE X 100

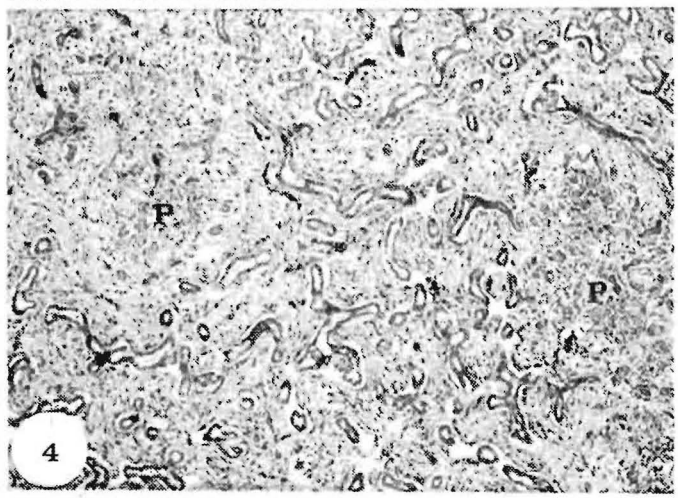
Fig. 5: Proliferating bile ducts embedded within fibrous connective tissue. Note the duplication of the epithelium (arrowheads) cytoplasmic debris within the lumen and separation of the epithelium from the underlying basal lamina. HE X 200

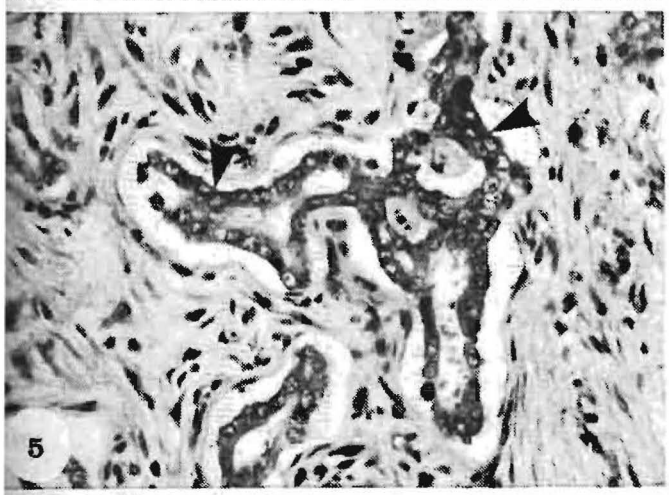
Fig. 6: Note the cytoplasmic inclusion within a bile duct epithelial cell (arrowhead). HE X 1000

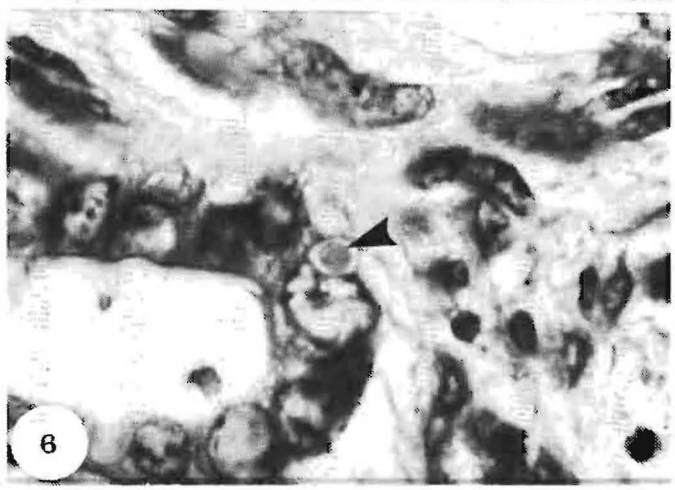
Fig. 7: Degenerative changes within the hepatocytes. A binucleated cell is arrowed. HE X 400

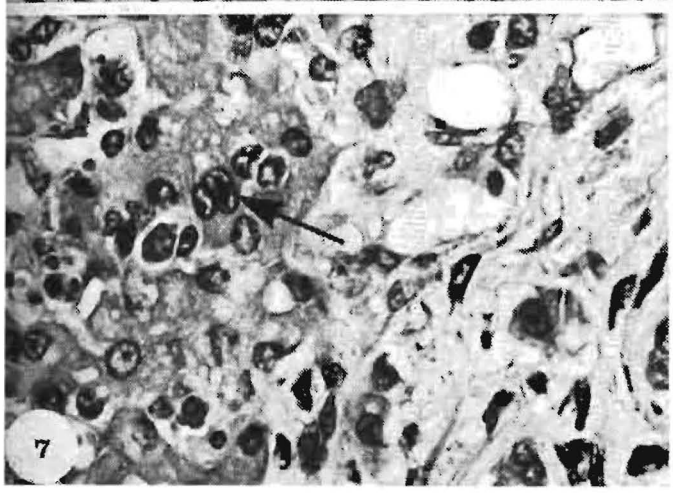
Fig. 8: Hepatocytes in various stages of degeneration. A multinucleated cell is arrowed. HE X 400

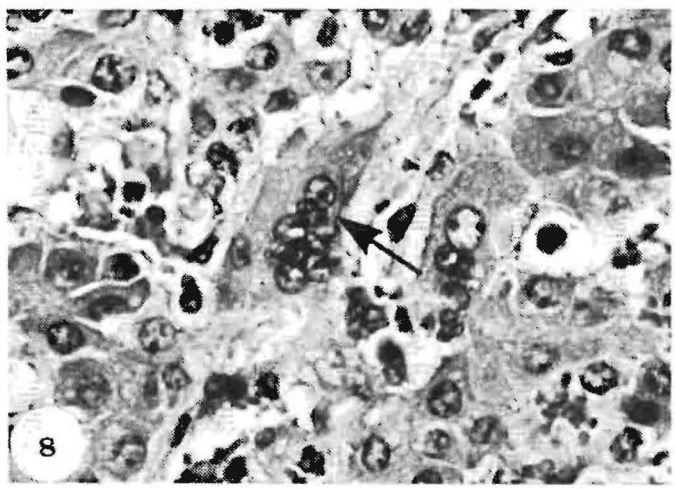












presence of tortuous subcapsular vessels probably represent an attempt to compensate for the portal hypertension which arose consequent upon the severe hepatic fibrosis[5].

In man, 2 types of biliary atresia (extrahepatic and intrahepatic) are recognised. Extrahepatic biliary atresia is progressive in nature and is associated with periportal and perilobular fibrosis, bile duct proliferation, cholestasis, pseudoxanthomatous transformation (lipofuscinosis), giant cell formation (in about 15% of cases), hepatocellular copper accumulation and ultimately cirrhosis[3]. Intrahepatic biliary atresia, on the other hand is seldom progressive in nature, being characterised by a paucity of intrahepatic bile ducts in the portal traids, occasional non-progressive periportal fibrosis and patchy pseudoxanthomatosis[3]. The histological lesions in this case conform to those of extrahepatic biliary atresia in man and is in agreement with the reported findings of a similar case in a foal[7].

The aetiology and pathogenesis of biliary atresia in animals is unknown. Whilst conjectural, 2 pathogenetic mechanisms are recognised in man, firstly, a congenital absence of bile ducts and secondly, postnatal destruction of bile ducts following chronic cholangiohepatitis. In so far as the latter is concerned, certain viral infections, namely rubella, varicella, reovirus and cytomegalovirus have been implicated[3].

Although a cholangiohepatitic pathogenesis cannot be excluded, it is our opinion that in this case, the biliary atresia was congenital in nature. A similar conclusion was arrived at by Ven der Luer & Kroneman in their recently reported study of biliary atresia in a foal[7].

A hepatopathy of foals with similar but not identical features to that of extrahepatic biliary atresia has recently been described[1,6]. This condition, however, involved foals 2 – 6 days of age and was associated with the feeding of a nutritional paste containing fermentation agents which presumably produced mycotoxins[1,6]. No such product was administered to this foal.

Extrahepatic biliary atresia should be considered as a pathological entity in foals 4 – 6 weeks of age showing signs of hepatic failure. Histopathological examination of the liver will confirm the diagnosis.

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CASE REPORT **GEVALVERSLAG**

DERMATOPHILUS CONGOLENSIS INFECTION IN CATTLE

R.F. HORNER*

ABSTRACT: Horner R.F. ***Dermatophilus congolensis* infection in cattle.** *Journal of the South African Veterinary Association* (1986) 57 No. 2, 121-123 (En). Allerton Regional Veterinary Laboratory, Private Bag X9005, 3200 Pietermaritzburg, Republic of South Africa.

The history, appearance and clinical course of a low incidence, chronic skin disease in beef cattle is reported. Calves were affected from 3 months of age and the condition persisted into adulthood. The infection was caused by *Dermatophilus congolensis* and resulted in severe crusting of the skin. Sheep were kept on the farm until 4 years ago. The method of diagnosis is discussed.

Key words: *Dermatophilus congolensis,* cutaneous streptothricosis, Senkobo disease, beef cattle.

INTRODUCTION

The bacterium, *Dermatophilus congolensis* is the cause of an acute or chronic skin disease in many species of domestic animals. The condition in cattle is also known as cutaneous streptothricosis or Senkobo disease. The infective phase of the organism invades cutaneous abrasions and initiates an inflammatory reaction characterised by the formation of a thick crust. The disease is essentially an exudative dermatitis with extensive scab formation[2]. The crusts adhere firmly to affected skin and are further held in place by hair fibres. Removal of crusts reveals a moist hyperaemic area. Heavy dandruff of the hair coat is common[2].

Transmission of the disease is by direct contact with infected animals or by indirect contact with mechanical vectors. Wetting of lesions appears to allow the release of infective zoospores[3]. Biting flies or ticks may be important in some areas as mechanical vectors, thus enabling foci of infection to begin at any point on the body. Damage to skin appears to facilitate the establishment of infection.

Infection is commonly seen along the back of cattle from the withers to the rump and extends to the mid lateral aspect[1]. Lesions may spread from a focus and occur anywhere on the skin. In young beef cattle lesions may begin on the rump probably due to the introduction of infection through skin abrasions caused by mounting[1].

The disease is common in tropical Africa especially under warm moist conditions but has been recorded from all continents. In tropical Africa the disease incidence in bovines may be as high as 10%[5].

CASE HISTORY AND CLINICAL FINDINGS

A chronic and persistant skin disease involving a small number of beef cattle from 3 months of age onwards, had been present on a Natal midlands farm for the past 4 years. During this period the condition had affected only 3 animals in a herd of 100. The herd was at pasture all year round. Each of the affected animals had been treated with a topical antifungal agent on at least one occasion. In addition to this treatment, simultaneous administration of parenteral antibiotics had been given by the owner. No improvement was noted in the condition of any animal.

One hundred and fifty dairy cows on the same farm were unaffected by this condition. Sheep had been kept on this farm until 4 years ago.

A 12-month-old Short Horn X bull in good bodily condition from the farm, was referred by a private practitioner to Allerton Regional Veterinary Laboratory for investigation of its skin condition.

A chronic dermatitis was evident over the rump and lumbar area which presented as focal dry crusts up to 30 mm in diameter with hair growing through (Fig. 1). On closer examination many smaller crusts could also be seen and in addition a coarse gravelly skin surface was felt when a hand was run over the coat. Severe dandruff was evident.

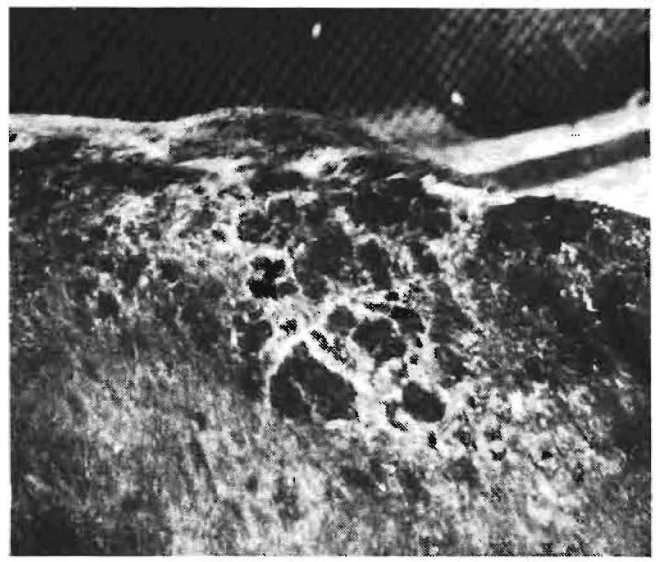

Fig. 1: Appearence of skin on presentation of bovine.

DIAGNOSIS

A blood sample was screened for packed cell volume, total red blood cell count and differential white cell count. Serum was evaluated for calcium, phosphorus, magnesium, copper, zinc, iron, potassium, total serum protein and albumin. All of these parameters were within the normal range for this laboratory.

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Parasitic and fungal investigations of skin scrapings were negative and initial bacteriological examination of similar material yielded mixed cultures. A skin biopsy was taken for histopathological examination from which Gram's-stained sections were prepared. These revealed the presence of branching filamentous organisms typical of *D. congolensis* as well as numerous micro-cocci in the surface crust and superficial epidermis (Fig. 2). After 3 weeks the animal was re-examined and a careful search made for the most recent and apparently most active lesions. Further skin scrapings and crusts were taken from these areas in order to positively identify the organism and confirm the diagnosis. Bacterial cultures isolated from these were streaked on Columbia blood agar medium containing 6% sheep blood and were incubated aerobically, microaerophillically in an atmosphere containing 10% CO_2 and anaerobically. The aerobic cultures yielded heavy mixed bacterial growth whereas the cultures grown in the microaerophillic environment favoured the growth of *D. congolensis*. This organism also grew in the anaerobic environment. *D. congolensis* was identified according to the description in Bergey's Manual[3].

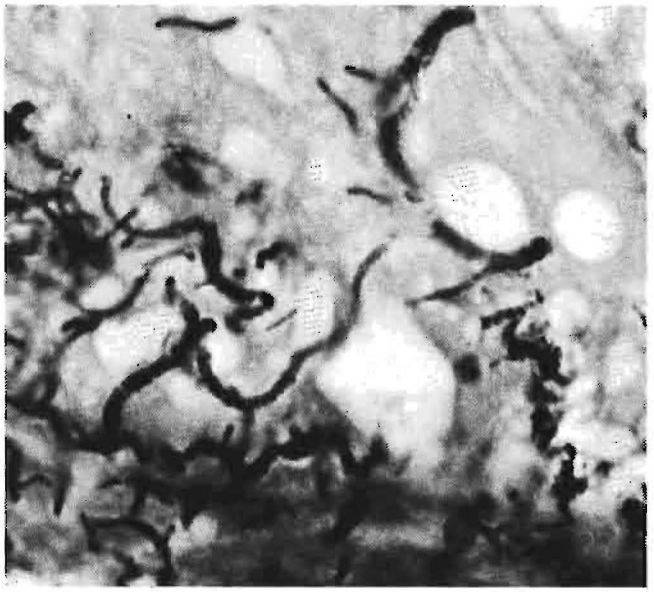

Fig. 2: Gram-positive filaments and cocci in skin biopsy.

TREATMENT

Treatment was undertaken using a combination of 70 000 units/kg procaine penicillin G and 70 mg/kg of dihydrostreptomycin sulphate, (Streptopen, Glaxovet) in a single intramuscular injection.

No improvement was noted and Gram's-stained smears made 3 and 4 weeks after treatment, were still positive for *D. congolensis*. Seven weeks later a second treatment regime was instituted which comprised daily intramuscular injections for 4 days of 30 000 units/kg of procaine penicillin G together with 30 mg/kg dihydrostreptomycin sulphate, (Streptopen, Glaxovet). One month later a mild improvement in the skin condition was noted. However, wet smears stained with Gram's stain still revealed filaments and cocci of *D. congolensis*. A third treatment one month later comprising a single intramuscular injection of a long acting oxytetracycline HCl, (Obermycin LA, Repvet) at 38 mg/kg also failed to cure the condition. Filaments and cocci were still present in Gram's-stained smears taken 5 weeks after treatment.

DISCUSSION

D. congolensis infection in cattle in South Africa appears to be uncommon. In sheep, however, the disease caused by the same bacterium is known as lumpy wool and is seasonally common especially in the higher rainfall areas of the country[4].

The low-grade chronic disease in beef cattle may be more common than is realised as professional help in diagnosis would possibly only be sought in acute or widespread severe cases. The appearance of the lesions seen in the cases described here somewhat resembled those associated with ringworm infection.

On this farm there had been a smouldering infection over at least 4 years. Once the animals were affected they remained so. The original source of infection may have come from sheep on the farm. In the absence of sheep since 1981, however, it seems that a chronic infection has persisted in a few beef animals. Topical treatments with iodine shampoo or antifungal washes plus antibiotic injections had been tried by the owner in the past, but with no success.

Diagnosis was achieved after collecting scabs and skin scrapings for laboratory tests and submitting a skin sample for histopathological examination. This latter procedure paved the way for a diagnosis in this case. It appears that with chronically infected animals careful selection of the most active lesions is important to enable demonstration of the bacterium. Initial bacterial cultures including those from the underside of crusts yielded mixed growth and thereby prevented a diagnosis from being made. With subsequent cultures from carefully selected lesions, the bacterium was isolated. CO_2 favoured the growth of *D. congolensis* and anaerobic conditions suppressed the growth of contaminants.

Roberts[6] states that a diagnosis can be readily confirmed in most cases with smears made from a suspension of finely chopped up scab material in a few drops of water. The smears stained with common bacterial stains are then examined for the presence of typical branching filaments dividing both transversely and longitudinally into thick bundles of coccoid forms.

Chronically infected animals are probably the organism's chief means of survival within a herd and act as reservoir hosts. Soil is unlikely to provide for proliferation or survival of the organism away from its animal host[6].

The hides of affected cattle may be unsaleable and in serious outbreaks individuals lose condition and die. However, in many herds there is no appreciable economic loss.

Treatment of the bovine by antibiotics alone was not successful for the three regimes tried. Combining a course of systemic antibiotic therapy with antibacterial shampoos plus manual scab removal may have been more successful. However, a practical and simple therapy that could and would be used on farms was sought here.

Chemotherapy may, however, be useful in chronically affected sheep. Roberts[6,7] found that a single intramuscular injection of 70 000 units/kg of procaine penicillin G together with 70 mg/kg of dihydrostreptomycin produced a 100% cure rate in infected sheep.

The success of this single treatment regime was borne out by work done on an infected ewe at this laboratory.

Crusts taken from the ewe were subjected to bacterial culture techniques and Gram's-stained smears as described previously. *D. congolensis* was demonstrated in this material.

A single intramuscular antibiotic treatment as described by Roberts[6,7] above resulted in a complete cure.

ACKNOWLEDGEMENTS

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CASE REPORT **GEVALVERSLAG**

THE PATHOLOGY OF PULMONARY ASPERGILLOSIS IN A PIGLET

J.W. NESBIT*

ABSTRACT: Nesbit J.W. **The pathology of pulmonary aspergillosis in a piglet.** *Journal of the South African Veterinary Association* (1986) **57** No. 2, 125-127 (En). Department of Pathology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

The pathology of pulmonary aspergillosis in a piglet is described. The dominant feature is the pneumonic process which is acute, focal, disseminated and necrotising to purulent in character. Fungal hyphae typical of the aspergilli are present within the lesions. The comparative aspects of this case and the primary invasive form of pulmonary aspergillosis in man are briefly enunciated.

Key words: Pulmonary aspergillosis, pig, pathology.

INTRODUCTION AND CASE REPORT

Aspergillosis refers to infection of man and animals by certain species of the ubiquitous fungus, *Aspergillus*[1,2,4-7]. Although members of the *A. fumigatus* group are most frequently involved, several other species belonging to the *A. flavus*, *A. niger* and *A. terreus* groups are occasionally incriminated[2]. The infection is not uncommon in birds but is of relatively rare occurrence in mammals[5-7]. Sporadic cases are on record in man and the domestic species. Reports of porcine aspergillosis are, however, extremely rare[1,7]. With regard to the latter species, the organism has been implicated in the aetiology of pneumonia[1,10] gastroenteriris[10], lymphadenitis[11], dermatitis[10], abortion[8] and, in one instance, generalised infection[1]. The infection is not contagious and is derived from an exogenous source; usually through inhalation of spores from a contaminated environment[1,4,7]. Therefore, whilst other organ systems may be affected, these are secondary to primary involvement of the respiratory tract in animals[1].

The purpose of this paper is to report on pathologic observations of pulmonary aspergillosis in a piglet.

Anamnesis

Five recently weaned piglets of indeterminate breed were acquired by the owner of a smallholding. Force of circumstance necessitated their being housed together with 30 chickens and 50 Muscovy ducks in the same enclosure. The piglets' diet consisted of kitchen and table scraps supplemented by occasional small quantities of milled maize. Approximately two weeks after their arrival all the piglets became anorexic and developed a severe diarrhoea concomitant with a moderate dyspnoea. Two animals succumbed within 24 hours, two after a period of 3 days whilst the last piglet survived in a largely comatose state for 5 days before death supervened. The latter animal was presented for post mortem examination.

Pathology

Other than a moderately outspoken cachexia, significant lesions were restricted to the lower respiratory and alimentary tracts. All the lung lobes were affected but not to the same degree; the diaphragmatic lobes evinced the greatest involvement. The lungs were diffusely greyish-red in colour and firmer in consistency than normal. In addition, numerous discrete spherical to oval nodules that varied in size from 1 – 4 mm, were distributed at irregular intervals over the surface and throughout the substance of the lungs. The nodules were either bright red or chalky white in appearance and in the latter instance, consistently surrounded by a narrow rim of hyperaemia. On palpation the nodules exhibited a moderate increase in consistency whilst, on the cut surface of the lung, plugs of yellowish-green pus exuded from some of the smaller elements of the bronchial tree.

A few superficial linear ulcers were evident in the fundic portion of the stomach which also exhibited marked mucosal and submucosal congestion. The entire intestinal tract, but the ileum in particular, manifested a similar intense mucosal and submucosal congestion. This was complemented by a moderate mesenteric and meningeal congestion.

The microscopic findings suggested an aetiologic diagnosis of miliary bronchopneumonia (of undetermined but possibly infectious or parasitic origin) complicated by terminal endotoxaemic colibacillosis.

Specimens of lung, liver, spleen, mesenteric lymph node and ileum were submitted for the isolation of potential pathogens. The results were disappointing as initial and subsequent attempts at both bacterial and fungal isolation yielded organisms construed as little more than contaminants.

Specimens of lung, mediastinal and mesenteric lymph nodes, liver, spleen, stomach, ileum and brain were collected and preserved in 10% buffered formalin for histopathological examination. Sections were routinely prepared and stained with haematoxylin and eosin (HE). Following examination of the sections, specific sections of the lung and stomach were selected and stained by the periodic acid-Schiff (PAS), Gram's (Brown-Hopps modification) and Gomori's methenamine silver (GMS) methods for light microscopy and the acridine orange method[9] for fluorescent microscopy.

Two distinct, although related, pathologic entities were encountered upon examination of the lung sections. The predominant reaction comprised an acute to subacute, focal, disseminated, necrotising to purulent bronchopneumonia of mycotic origin (Fig. 1 – 4) which

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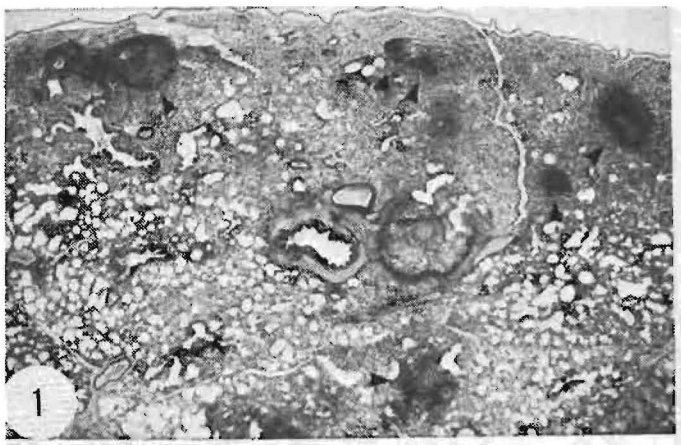

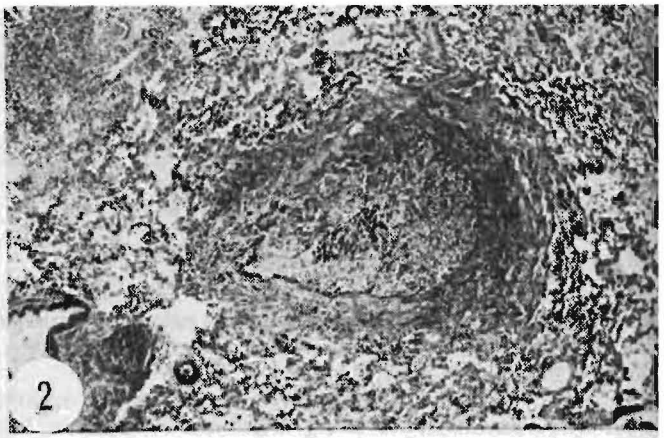

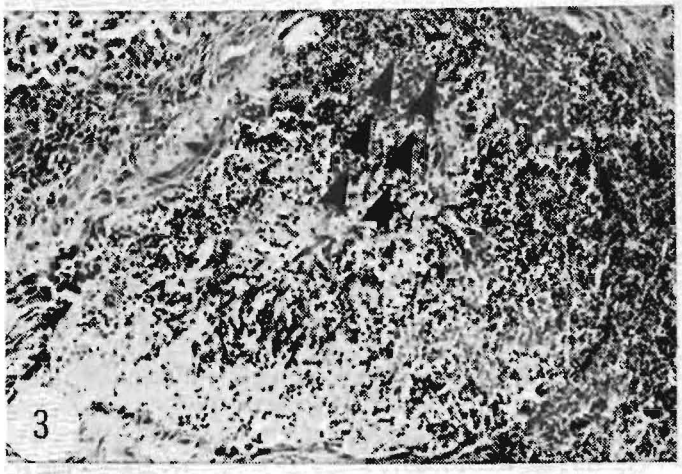

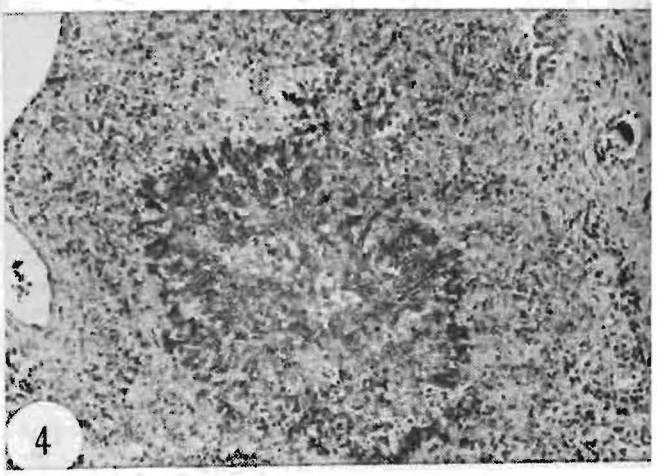

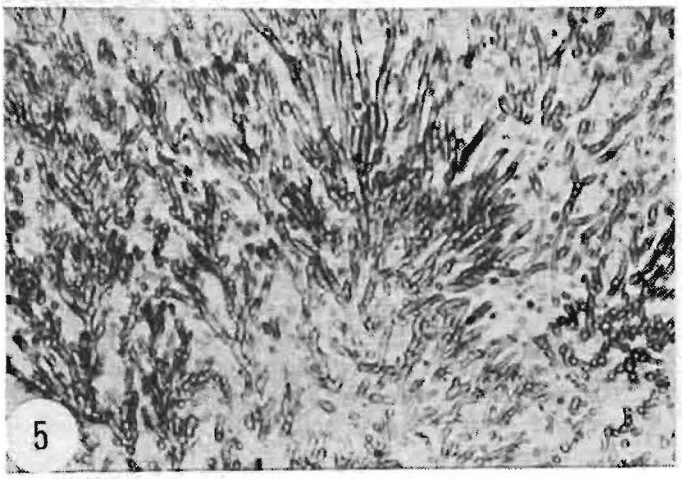

Fig. 1: Disseminated foci of bronchopneumonia (arrowed Gram's-stain.

Fig. 2: Early lesion. Acute, segmental, necrotising bron chiolitis. PAS X 40

Fig. 3: Early lesion (higher magnification of the previou figure). Note the segmental necrosis with orientation o the penetrating hyphae in the same direction (arrowed PAS X 200

Fig. 4: Advanced lesion. Radiation of proliferating hypha from the central mycelial mass in the necrotic core Note the greater affinity of the peripheral hyphae fo the stain; indicative of zonation of hyphal growth. PA X 200.

Fig. 5: Note the acutely angled dichotomous branching of th septate hyphae characteristic of the aspergilli. GMS 400

was accompanied by a diffuse, interstitial, essentially plasmacytic pneumonia of moderate severity in the remainder of the lung. Stages in the development of the pneumonic foci were evident. Early lesions (Fig. 2 & 3) were restricted to the bronchioles whilst the more advanced lesions (Fig. 4) involved the parenchyma. In the former instances, proliferating fungal hyphae were seen penetrating the walls of the affected bronchioles. The hyphal filaments all tended to be orientated in the same direction. Penetration was associated with segmental necrosis of the full thickness of the bronchiolar wall and was accompanied by a leukocytic, predominantly neutrophilic, infiltration into the region. The lumen of the affected bronchioles were frequently plugged with an exudate consisting of dead and dying neutrophils, macrophages, tissue debris, mucus and fungal elements. On the other hand, the more advanced lesion consisted of a central area of coagulative necrosis surrounded by a zone of acute fibrinopurulent inflammation and atelec tatic pulmonary tissue. Proliferating fungal hypha radiated from a tangled mycelial mass within the centra area of necrosis (Fig. 4) to form a "Sun burst" or ac tinomycetoid pattern. Although the causative organisr was readily discernible in HE sections, the detailed mor phology was highlighted in sections stained with PA (Fig. 2 – 4) and GMS (Fig. 5) for light microscopy. Th fungal elements were similar in both the early and ad vanced lesions. The hyphae exhibited uniformity o width, septation and acutely angled dichotomou branching. In addition to a light green (fluorescent staining, these features were also apparent in section stained with acridine orange and examined under blu light by fluorescent microscopy. In some PAS-staine preparations, and particularly with regard to the large fungal colonies, the peripheral hyphae demonstrated greater affinity for the stain than the central myceli

mass (Fig. 4); an indication of zonation of hyphal growth. The interstitial tissues of the remaining, relatively unaffected, portions of the lung were thickened due to an infiltration of predominantly plasma cells accompanied by occasional lymphocytes, macrophages and neutrophils.

Examination of sections of the other tissue collected, served largely to confirm the macroscopic findings: relatively mild reactive hyperplasia of both the mediastinal and mesenteric lymph nodes; mild hepatic and splenic congestion; superficial gastric ulceration of indeterminate cause together with marked mucosal and submucosal congestion; marked mucosal and submucosal congestion of the ileum; and, moderate meningeal and cerebral congestion.

DISCUSSION

In spite of the unsuccessful attempts at laboratory isolation, a diagnosis of pulmonary aspergillosis was deemed appropriate in this case. There is no doubt that the pulmonic lesions were directly attributable to the associated mycotic infection. Although cultural studies are considered essential for species identification[2 4 7] the morphologic features and staining qualities of the aspergilli in histologic sections are sufficiently characteristic to justify their positive identification on a generic basis[1 2 4 7-9]. Thus, the septate hyphae of constant width and the acute angulation of the dichotomous branches together with the light green fluorescence are typical, almost unique, light[1 2 4-7] and fluorescent[9] microscopic attributes peculiar to this genus. Moreover, the growth characteristics (manifested by development of an actinomycetoid pattern and zonation of hyphal growth in the larger fungal colonies) also provide supportive evidence as to their identification[2].

According to the histopathologic evidence it would appear that the infection was derived by inhalation. An initial bronchiolar invasion preceded the subsequent parenchymal involvement. The invasive process is presumably facilitated by the concomitant liberation of a purported necrotizing toxin[2] together with active fungal proliferation[2 4]. Whereas bronchiolar penetration was segmental with orientation of the fungal hyphae in the same direction, the parenchymal reaction was focal and the hyphae tended to assume a radiating or actinomycetoid pattern. Necrosis was extensive in the parenchymal lesions and was presumably the function of the purported endotoxin[1 3]. Whilst the focal reaction is dominant, the related diffuse plasmacytic interstitial reaction is probably a reflection of a burgeoning immune response to the invading microorganism. Dissemination to other tissues, notably the meninges, brain, heart, kidneys, skin and bone, is apparently of rapid occurrence in both man[2 4] and animals[5-7]. Metastasis is invariably via the haematogenous route. This is due to the propensity of the mycelium for vascular invasion and development of a thrombotic angiitis[2 4-7]. No specific mycotic involvement was evident in the pulmonary vasculature in this case; further attestation of the acute nature of the infection. The pneumonic lesions, however, bear a close resemblance to descriptions of pulmonary aspergillosis in the young of other animal species[1 5-7 10]. Other than the gastric ulceration which may be considered incidental, the congestion present in the other organs has also been described in previous investigations[1 3 5-7 10] and is probably ascribable to the action of the purported endotoxin[1 3].

The nature and extent of the pulmonic lesions, when coupled to the morphologic and growth characteristics of the causative organism as well as the clinical course of the illness, is highly suggestive of an acute, ongoing, active, disseminating phase of infection[2 4]. In this respect, the disease in the subject of this report appears analagous to the primary invasive form of pulmonary aspergillosis in man[2]. Aspergillosis is not contagious and the source of infection is exogenous[1 2 4-7]. Frequently, secondary infection may arise as a result of preceding congenital or acquired pulmonary pathology or following immunosuppression related to continued antibiotic and/or corticosteriod administration. However, it may occur in the absence of other demonstrable predisposing factors; as in this case. Continuous or repeated environmental exposure to heavy spore and/or conidial loads may overwhelm the normal pulmonary defence mechanisms and give rise to a fulminating pneumonic process. Although speculative at best, two factors may have contributed to the development of pulmonary aspergillosis in the piglet. The abundance of aspergilli in the avian environment is well recognised[1 4-7 10]. Thus, confinement of the piglets to an enclosure usually reserved for chickens and Muscovy ducks may have led to excessive environmental exposure. Secondly, the contribution of the stress of a new environment together with a poor nutritional status must be acknowledged.

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