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CONTENTS/INHOUD

Bayer Handycure

Die Handige Reeks Verpakkings vir die Klein Boer.

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

RINTAL Rondewurmmiddel met 27A'S vir skape en bokke.
I.C.I. LEWERSLAK MIDDEL Vir beeste en skape.
DYLOX INSPUITBAAR Rondewurmmiddel vir beeste.
TRAMISOL Rondewurmmiddel vir beeste en skape.
SULPHAMEZATHINE 16% Middel vir koksidiose kalwers, lammers, hoenders en konyne.
OXY-VET Bree spectrum antibiotikum.
BACDIP NF2 Dipstof vir beeste, skape, honde en perde.
BACDIP AEROSOL Handige spuit vir lastige bosluis kolle.
DELTRAM Rondewurmmiddel vir skape en bokke.
LINTEX M Dood lintwurms en onvolwasse peervormige slak in kalwers en lammers.
BABESAN Roolwater middel vir beeste.
TIGUVON SPOT-ON Dood luise op beeste.
HIBITANE STEEK PILLE Vir baarmoeder infeksie by beeste en skape.

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ENZOOTIC ICTERUS – A FORM OF CHRONIC COPPER POISONING

G.F. BATH

ABSTRACT: Bath G.F. **Enzootic icterus – A form of chronic copper poisoning.** *Journal of the South African Veterinary Association* (1979) **50** No. 1 3 (En) Private Bag X528, 5900 Middelburg, Rep. of South Africa.

The findings of a recent investigation into the epizootiology, course, symptomatology, gross pathology, chemical pathology and prevention of Enzootic Icterus are presented and the findings of previous investigations on these and other aspects are re-evaluated. Enzootic Icterus and chronic copper poisoning are shown to be identical in all fundamental features. Both diseases are characterised by sudden onset, high mortality, icterus, anaemia, haemoglobinaemia, methaemoglobinaemia, haemoglobinuria and deeply pigmented kidneys. There are no described microscopic features on which the diseases can be differentiated. Very high levels of copper in the blood, liver and kidneys are found in both diseases. In the present work 36 liver specimens had a mean copper concentration of 701 μg/g. Of 31 kidney specimens no values under 10 μg/g were found while the mean was 107 μg/g. Prevention of Enzootic Icterus by salts of molybdenum given orally confirms the identity. No close relationship with Geeldikkop is found other than the probability that the two diseases may act synergistically. Interpretation of existing knowledge of mineral levels of natural grazing in the enzootic region shows that the levels can account for the presence of chronic copper poisoning, although more investigation is required.

INTRODUCTION

Although enzootic icterus (EI, geelsiekte) has been the subject of numerous studies, either direct of indirect, its cause has remained the subject of considerable controversy[8–11 13 21 26 27 39–41 45–47 58]. Brown and his co-workers considered that EI and geeldikkop were different manifestations of the same disease condition, possibly subclinical selenium poisoning[8–11 13 45–47] but this has not been satisfactorily confirmed[39–41 45 47]. No experimental cases of selenium poisoning resembling either EI or geeldikkop have been produced. Instead, later work has shown that geeldikkop is distinct in both its aetiology and pathology[62].

Earlier workers quoted by Brown[8 10] noticed that close similarity between EI and described forms of chronic copper poisoning. Notwithstanding these similarities, Brown came to the conclusion that copper poisoning was not involved in the aetiology of the disease[8–11 46]. However, the generally very high liver copper levels found by him and others[26 27 40] in cases of EI were not satisfactorily explained. Erasmus[26 27] has demonstrated that it is extremely unlikely that they could have been the result of low grade haemolysis, and noted that cases of EI had high levels of liver copper while those in geeldikkop cases were generally normal[26 27]. The constant presence of high liver copper levels in EI remains unexplained except on the basis that the disease is a form of chronic copper poisoning. Previous conflicting findings on geeldikkop and EI have led to a complete reappraisal of ovine diseases involving icterus and photosensitisation.

Numerous aspects of EI have been reviewed and investigated between 1972 and 1977. Since Brown[8–11] considered that geeldikkop and EI were different forms of the same disease condition, an immediate difficulty was obvious. If all diseases involving icterus and photosensitisation in the enzootic region were to be investigated on the assumption that they were aetiologically linked, a very confusing picture might have emerged. It was accordingly decided that to qualify for the description of EI, the presenting syndrome had to have at least some of the diagnostic criteria by which EI is generally recognised[20 21]. These include icterus, anaemia, haemoglobinaemia, methaemoglobinaemia, haemoglobinuria and deeply pigmented dark brown to black kidneys.

Since the prime aim of this investigation was to try to establish beyond reasonable doubt whether or not EI is a form of chronic copper poisoning, most investigation was directed specifically to this end. This paper records the findings in the present investigation and compares them to previous findings. Certain previous work is re-evaluated in the light of the present investigation.

EPIZOOTIOLOGY

EI occurs in sheep originating from a large area of the Cape Province and the southern Orange Free State. The approximate known extent of the main enzootic area is indicated in the accompanying map (Fig. 1). Areas outside this main area may experience the disease and conversely many farms within the area have never reported cases. The southwestern part of the area is most severely and frequently affected, but severe outbreaks may occur almost anywhere in the area depending on local circumstances. It is sometimes striking that a particular farm may suffer regularly from the disease while neighbouring farms are apparently unaffected. More commonly a whole region is susceptible to outbreaks. A map showing actual incidence on specific farms would thus be a patchwork throughout the general region.

The enzootic area generally lies above 1000 m above sea level and geologically is part of the Dwyka, Ecca and Beaufort Series which consist mainly of shales, sandstones and doleritic intrusions[11 13 39]. A constant feature of farms where the disease regularly occurs is the presence of these doleritic intrusions, although they are also found on many unaffected farms. The topography of the area varies from fairly mountainous regions to undulating, broken veld and flat, almost featureless stretches of country. Most affected farms include mountainous or hilly aspects.

Rainfall is generally low and falls mainly in the second half of summer. As would be expected in a high inland region, temperatures vary considerably both daily and annually and very cold winters followed by very hot summers are the rule.

It is evident from the accompanying map that the enzootic area covers a number of veld types[1]. As a general rule, farms on which EI is encountered have been subjected to bad veld management practices, although this is not always the case. Well managed farms seem to suffer less severely from outbreaks.

Outbreaks of the disease are brought on by a variety of factors, which all involve a form of stress. In the first

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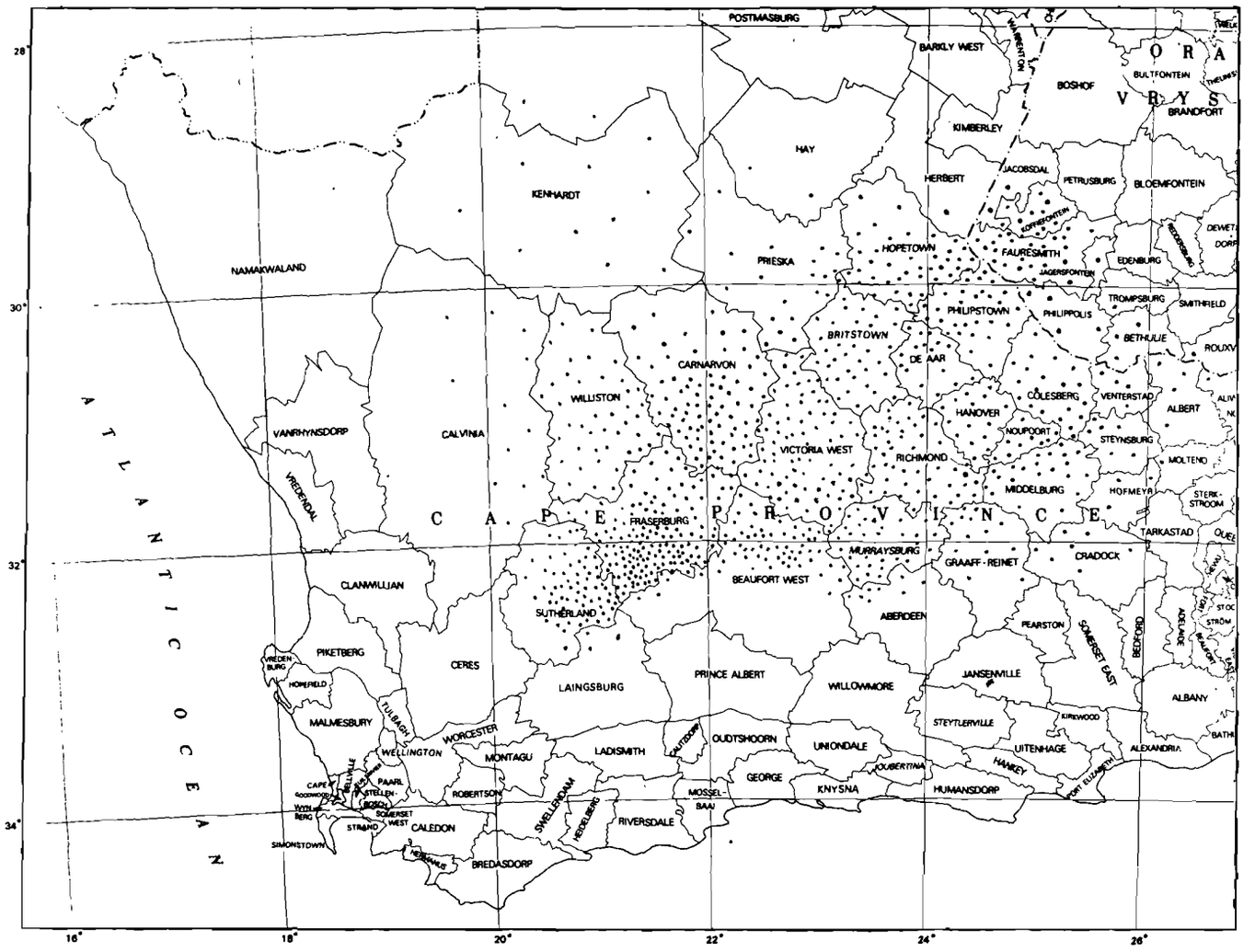

Fig. 1. The approximate extent of enzootic icterus. Points do not represent actual outbreaks but rather the incidence in each district.

instance transportation or trekking, especially when extending over several days, is a potent trigger for the disease. Under these circumstances EI is found throughout South Africa in sheep which emanate from the enzootic region. Within the region the most common factor is poor nutrition, brought on by deterioration of the veld in late winter and early summer when little rain falls and dry westerly winds blow. It is generally agreed by farmers that most cases occur between September and December but often large outbreaks can continue into March. Outbreaks are more common and severe in drought years than in good years. Farmers are almost unanimous that during such outbreaks, good rains bring a halt to further deaths, although many cases can still occur within the first two weeks after rain has fallen. The latter phenomenon is probably related to a sudden change in grazing and also happens when sheep are brought onto lush pastures. In these cases an apparent improvement in nutrition is responsible for initiating the disease.

Other stress factors, particularly when in combination with each other or with poor nutrition, can cause sporadic outbreaks at any time of the year in enzootic areas. These factors include sudden changes in weather, dosing, dipping, inoculation, shearing, lambing, pregnancy and lactation. Frequently sheep are driven for several kilometres and held for prolonged periods for shearing, dipping or dosing before being returned to their pastures. Sometimes other diseases act as a stress factor and precipitate an outbreak. In all these cases at least an element of digestive disturbance and temporary or relative undernutrition seems to be the common factor. In some outbreaks no obvious form of stress can be identified.

The disease occurs in all breeds of sheep which are kept in the region. Because the Merino or its variations and crosses is still the dominant breed in the region, most cases occur in animals of this type. British mutton breeds like the Hampshire Down seem relatively more susceptible to EI although few are found in the enzootic region. On farms where both Dorpers and Merinos are kept, most farmers agree that Dorpers are relatively more likely to develop the disease and die more readily and rapidly from its effects. Goats are very seldom involved and no cases have been seen in cattle or other domestic species.

Rams, wethers and ewes can all contract the disease. Ewes are most frequently affected, probably partly because they are kept for a long time for breeding purposes and are more subject to stress when pregnant, lambing or lactating. Because of their value, rams are often given special care and feeding, which appears to render them less susceptible. In wethers, there is an apparent lower incidence compared to ewes but this is

probably due to their smaller number, comparative youth when sold, and the absence of additional stress related to breeding which is present in ewes.

A characteristic feature of EI is that almost without exception only the older sheep of the flock are involved. The disease is extremely rare in sheep under six tooth while old worn-mouth animals constitute the overwhelming majority of clinical cases and deaths.

In most outbreaks the animals have lost some condition before the disease begins. This is probably the result of poor grazing and undernutrition as already outlined. While sheep in poor condition are more likely to develop the disease, those in good condition when they do develop EI seem more likely to die suddenly.

Morbidity varies considerably depending on the epizootiological factors involved. Usually it is between 0,5 and 5 % of the total flock but may reach 10 and even 20 %. A number of subclinical or inapparent cases may also be present. Mortality is usually between 90 and 100 % of those obviously clinically affected.

Total losses in the region are difficult or impossible to determine with any great accuracy. The area is huge, there are few veterinarians or stock inspectors, and many farmers are not aware of the disease or do not report outbreaks. However, the enzootic region contains over five million sheep according to the 1976 stock census. Judging by the number of farmers who report losses of between 20 and 200 sheep in different years, the total loss in the region must be considerable. Probably at least a thousand sheep die annually but this figure rises to well over ten thousand in bad years, depending on various factors outlined previously. Additional economic losses are sustained in the loss of production in survivors of milder attacks and there are indications that sheep on EI-prone farms may suffer more from other unrelated diseases, especially when these act as a precipitating stress factor for severe or mild forms of EI.

The findings on epizootiology in the present work are largely in agreement with previous workers[10 11 21 58].

COURSE OF THE DISEASE

In EI it is of prime importance to appreciate that the stage the disease has reached in individual animals when examined considerably influences the symptoms, necropsy findings and chemical pathological characteristics encountered.

There is firstly a preclinical or incipient stage which is only detected in apparently normal sheep in the flock on close examination and, more commonly, by blood analysis. Sometimes these preclinical cases may pass over into inapparent, very mild haemolytic attacks which are generally not noticed by the farmer. Normally, however, the preclinical phase is very rapidly followed by a severe haemolytic episode which is so characteristic of the disease. Again, sometimes this crisis may be fatal within hours but usually the animal will survive for between twelve hours and four days of becoming obviously affected. During this period further haemolytic crises may occur. In some cases sheep survive well beyond four days and may even recover completely over a period of thirty days or longer. Often, however, they eventually die either from further haemolysis or secondary complications like nephrosis.

This clinical spectrum forms an infinite series of gradations between mild or inapparent forms, the characteristic severe peracute and acute forms, and chronic survivors of mild or severe episodes. If additional severe or mild haemolytic crises occur at any stage, the picture will naturally be further complicated. Generally, the duration of illness is inversely related to the severity and rapidity of haemolysis. It is further influenced by the breed, age and condition of the flock as well as the particular stress factors involved in precipitating an outbreak. Although clinical EI may run an acute or chronic course and may be accordingly more or less severe in individual animals, these differing forms should not be interpreted as completely distinct entities but rather as representative forms within a large spectrum. When mention is made of these forms the description should be seen in this light.

Although this description is essentially the same as previous workers[20 21] completely distinct acute and chronic forms, without intermediate forms, are not recognised.

SYMPTOMATOLOGY

In many cases the course of the disease is too short for symptoms to be observed under field conditions. Symptoms observed depend very largely on the time interval between initial haemolysis and examination, as well as the severity and number of haemolytic episodes. The breed, age, condition, breeding status of ewes, as well as the particular stress factors involved in an outbreak and the presence of other intercurrent diseases in individual sheep also modify the symptom complex. The presence of other diseases would of course severely complicate a description of characteristic symptoms; their influence in cases of EI is accordingly discussed separately.

Although the majority of affected animals are in poor condition when first showing obvious signs of EI, many are in fair or even good condition. As the disease runs its course survivors become progressively more emaciated. Typically sheep become apathetic and listless and frequently stand or lie alone for long periods in one position with the head lowered. Affected sheep often have an unsteady gait and lie down readily. When driven they lag behind the flock, frequently fall down and are unable to rise for a time. Anorexia is usually total. Nasal discharge, conjunctivitis and keratitis are sometimes seen in chronic survivors.

Respiration and heart rate are rapid even at rest but are most obvious after slight exercise. The pulse is strong initially but weak terminally. Body temperature varies considerably depending on the stage of the disease, ambient temperature and degree of exercise. Initially it is high and falls terminally. The faeces are usually hard and dry, dark brown in colour and may be covered by mucus which is sometimes tinged with blood. In some instances diarrhoea may be present. Palpation of the rumen usually reveals atony.

The most characteristic signs of EI are seen in the mucous membranes, urine and blood. Visible mucosae may be pale to very pale depending on the duration of symptoms. In peracute cases there is little sign of anaemia, while the chronic survivors are characterised by severe pallor. On this pallor a very characteristic dirty brownish, greyish or greenish jaundice is usually superimposed. Like the anaemia in peracute cases, jaundice is sometimes slight initially, increases to a height in subacute cases, and falls to become slight or absent in

chronic cases. The characteristic dirty discolouration is usually only seen in the first few days. It is difficult to draw blood because of collapse of the veins. In acute cases the blood is very watery and commonly a dark brownish-red colour. The urine is a very characteristic reddish-brown to almost black colour in acute cases, while it becomes progressively more normal in chronic survivors. Haemoglobinuria can be suspected if dark, damp patches are seen on the ground where an affected sheep has been standing.

The symptomatology as described here is generally in agreement with previous workers[20] [21].

GROSS PATHOLOGY

Necropsy findings are modified by the same factors which influence symptomatology. Carcasses are usually in poor condition and often emaciated in chronic cases but poor condition is not invariably present in acute cases. Icterus is usually a prominent feature, though it may be absent in animals which die in the peracute or chronic stages. Sometimes icterus only becomes apparent some time after the carcass has been opened. It is usually a characteristic dirty brownish, greenish or greyish yellow but can also be bright yellow. Carcasses commonly contain scanty blood which clots poorly and is often a characteristic dark brownish red colour. Anaemia together with emaciation is the most striking general feature in chronic cases.

Skeletal musculature is usually dark red. The lymph nodes are swollen and moist in acute cases and atrophied in chronic cases. The medulla is characteristically pigmented a brownish-green colour. The spleen is usually enlarged and the contents are dark red to brown and often pulpy.

Gastrointestinal stasis is common but not invariably present. The walls of the gastrointestinal tract are usually atrophic and the contents are hard and dry especially in chronic cases. Sometimes blood or bloody brownish contents are found in the intestines. Haemorrhages may be found in the wall of the digestive tract and elsewhere in the carcass. The heart is usually pale and flabby. Lung oedema, hydropericard, hydrothorax and ascites are common and the fluid is usually brownish or yellowish.

In peracute cases the liver is reddish, while in acute and subacute cases it is a dirty brownish yellow to khaki colour which gradually returns to normal in chronic cases. The consistency is initially friable but becomes progressively firmer in later stages of the disease. Lobuli are prominent in acute stages and are a pale yellow colour. The liver is initially swollen, smooth and tense but progressively becomes smaller and more irregular in chronic cases. The gall bladder becomes progressively distended with thick, greenish bile.

The most constant and characteristic lesions are seen in the kidneys, which are always pigmented. Initially (in the peracute form) pigmentation can be relatively slight and the cortex is irregularly mottled by dark red or brown areas which extend into the medulla. Much more commonly the entire kidney is deeply coloured a dark reddish-brown, grey-brown or black. Sometimes faint irregular mottling between these colours can be seen on close examination of the surface of the cortex. The colours extend as radial streaks into the medulla, which is often difficult or impossible to differentiate from the cortex. In this classical acute phase, which constitutes over 90 % of deaths, the kidney is also grossly swollen and very friable. In chronic cases which relapse and die or are slaughtered, lesser changes are seen. The kidneys appear dark greyish brown and are not grossly swollen. If urine is present in the bladder, it is reddish to brownish-black in the acute phase, while in chronic cases it may appear normal. Usually carcasses decompose very rapidly and have a very unpleasant smell within hours of death.

Gross pathology of EI reported previously[20] [21] is in agreement with present findings in most respects.

CHEMICAL PATHOLOGY AND HAEMATOLOGY

Since the present investigation was largely intended to establish whether EI is essentially a form of chronic copper poisoning, the blood, organ and urine tests carried out were specifically selected to this end.

Standard methods were used to determine the haematocrit (Ht), total plasma proteins (TPP), blood urea (BU), conjugated (CBil) and total plasma bilirubin (TBil), plasma glutamic oxalacetic transaminase (GOT), plasma iron (PFe), plasma copper (PCu) and blood copper (BCu). The minerals in blood, plasma and urine were determined by atomic absorbtion spectrophotometry. In some cases blood haemoglobin, plasma haemoglobin and creatinine were also determined in blood samples. Liver and kidney specimens were preserved in 10 % formalin and then wet ashed for copper and iron analysis by atomic absorbtion spectrophotometry. Organ values are therefore on a wet basis, which are approximately one third to one quarter of those which would have been obtained on a dry basis[3] [10]. Urine was examined for the presence of blood, protein, glucose and ketones, and the pH reaction was determined by the use of semiquantitative strip tests (Labstix, Ames). Haemoglobin, bilirubin, iron and copper were determined by methods adapted from blood analysis.

Blood was taken from 24 animals clinically suffering from the disease in 11 outbreaks; their blood pathology is shown in Table 1. In 10 outbreaks, random blood samples from between ten and twenty sheep from the rest of the flock were taken. Out of 172 samples, 22 showed changes consistent with the early outset of disease. In two of these at least, there was evidence of a previous episode. The blood pathology of the 22 cases is shown in Table 2. A further 27 sheep showed milder disturbances of chemistry, particularly high plasma and blood copper levels. Specimens of the liver and kidney for mineral analysis, were taken from freshly dead subjects or those slaughtered for autopsy. Altogether 36 liver and 31 kidney specimens were examined. The individual analyses are shown in Table 3.

Since there was no way of accurately determining the stage of the disease, its severity or the number of previous haemolytic episodes in individual sheep when they were first seen and bled and before the results of blood tests became available, no attempt has been made to classify individual cases further in the tables. Later, determination of haematocrit readings and TPP, GOT, PFe, PCu, BCu and bilirubin levels could be used to assess the probable stage of the disease in individual sheep.

A critical evaluation of the blood picture reveals that preclinical cases (those in which blood abnormalities were found after bleeding a random sample of the

Table 1: CLINICAL CASES

Case No.	Ht	TPP	BU	GOT	PFe	BCu	PCu	C Bil	T Bil
73/111-1	0,13	93	3,3	133*	166,5	29,4	21,5	15,4	30,8
73/844-1	0,09	70	20,6	120*	–	–	37,1	6,8	10,3
73/844-2	0,14	73	31,5	135*	–	–	62,5	10,3	32,5
73/844-3	0,12	76	7,8	98*	–	–	29,4	0	8,6
73/844-4	0,12	64	2,5	111*	–	–	28,6	–	–
73/844-5	0,12	85	23,1	100*	–	–	25,1	–	–
73/844-6	0,12	75	19,1	150*	–	–	27,6	1,7	13,7
73/844-7	0,48	90	13,3	120*	–	–	101,0	–	–
75/942-1	0,15	90	8,0	48	64,8	43,6	48,7	0	46,2
75/1023-1	0,18	73	27,2	190	31,3	51,5	59,0	25,7	58,1
76/372-1	0,25	75	2,2	570	86,5	27,0	34,9	–	–
76/372-2	0,33	75	1,5	240	18,3	18,8	17,0	–	–
76/448-1	0,13	112	–	190	147,5	52,8	51,3	–	–
77/638-1	0,19	71	10,3	190	36,5	70,7	70,7	–	–
77/641-1	0,29	82	2,7	110	63,9	51,8	89,5	0	6,8
77/641-2	0,09	83	37,4	190	36,5	77,7	33,0	0	6,8
77/668-1	0,08	64	13,6	110	36,5	61,2	61,2	0	8,6
77/668-2	0,12	104	11,8	340	100,4	75,4	103,6	8,6	8,6
77/668-4	0,37	74	2,2	670	54,8	75,4	61,2	0	15,4
77/668-5	0,22	101	4,0	240	91,3	433,3	51,8	10,3	10,3
77/668-6	0,26	74	18,6	160	36,5	80,1	56,5	1,7	1,7
77/699-1	0,17	68	35,5	240	23,3	61,7	57,3	0	8,6
77/699-2	0,14	82	13,6	80	46,5	68,3	73,8	8,6	20,5
77/784-1	0,14	83	15,8	280	91,3	166,4	125,6	17,1	27,4
	1/1	g/ℓ	mmol/ℓ	mU/mℓ	µmol/ℓ	µmol/ℓ	µmol/ℓ	µmol/ℓ	µmol/ℓ

*GOT levels in IU/dℓ

Table 2: FLOCK CASES

Case No.	Ht	TPP	BU	GOT	PFe	BCu	PCu	C Bil	T Bil
73/1219-1	0,38	71	4,6	110	9,1	364,2	28,3	–	–
73/1219-2	0,36	74	2,7	137	18,6	54,8	61,4	–	–
73/1219-3	0,40	75	3,3	117	33,3	174,6	33,0	–	–
73/1219-4	0,32	70	2,7	79	42,6	66,9	39,1	–	–
73/1219-5	0,33	75	2,7	62	18,4	78,7	22,8	–	–
73/1219-7	0,36	68	2,5	62	27,9	200,3	22,8	–	–
73/1219-9	0,30	67	3,3	103	37,6	51,7	21,7	–	–
74/965-15	0,37	80	11,0	35	102,7	85,6	83,2	3,4	27,4
74/965-18	0,37	98	10,8	45	177,6	359,5	208,3	90,6	143,6
74/965-19	0,28	99	4,6	48	123,7	215,6	148,2	18,8	37,6
74/988-1	0,33	91	4,3	124	98,6	180,6	94,5	0	49,6
74/988-2	0,19	88	3,7	122	90,8	77,7	86,8	12,0	44,5
74/988-3	0,35	71	2,5	112	40,1	32,7	71,6	–	–
74/988-7	0,35	71	1,8	124	36,7	30,5	67,8	0	6,8
74/988-11	0,37	80	2,7	88	43,3	43,3	86,8	0	3,4
74/988-12	0,30	90	3,0	91	86,8	314,0	94,5	15,4	99,2
75/942-13	0,34	74	2,5	40*	47,8	277,9	14,8	–	–
75/942-14	0,32	69	1,5	40*	22,7	18,1	75,4	–	–
75/942-20	0,33	87	4,3	80*	82,5	171,1	91,7	1,7	8,6
75/1138-11	0,18	83	2,2	80*	24,2	24,0	65,2	–	–
76/618-12	0,35	80	1,8	400*	86,3	242,1	47,1	0	12,0
76/618-13	0,40	92	1,8	240*	42,8	200,2	89,6	8,6	20,5
	1/1	g/ℓ	mmol/ℓ	IU/dℓ	µmol/ℓ	µmol/ℓ	µmol/ℓ	µmol/ℓ	µmol/ℓ

*GOT values in mU/mℓ

flock) show elevated GOT, PCu and BCu levels. Haematocrit readings may be lower or normal in these cases. TPP is often raised, indicating haemoconcentration which can mask the drop in haematocrit. BU is invariably normal unless previous haemolytic episodes have occurred (cases 74/965–15 and –18). PFe is usually normal but is raised at the onset of haemolysis or following previous crises. In clinical cases, the onset is characterised by moderate to marked elevation of PCu and a severe elevation of BCu. PFe rises a little later and does not reach the same extreme peaks as copper. At the same time there is a rapid fall in the haematocrit reading and much free haemoglobin appears in the plasma. Often anaemia may be masked by haemoconcentration. GOT and TPP levels may be elevated or normal. Bilirubin levels are elevated in most cases.

Table 3: LIVER AND KIDNEY MINERAL ANALYSIS (μg/g WET MASS)

	Liver		Kidney	
Case	Cu	Fe	Cu	Fe
73/844	1047	122	11	296
73/972	836	67	46	284
	765	85	167	206
73/1009	979	148	46	286
	979	170	17	744
74/965	563	102	49	229
	1428	48	105	118
	188	105	151	205
	1428	55	–	–
	704	143	–	–
74/988	734	77	123	146
	842	74	279	393
75/942	1150	92	265	891
	1250	168	–	–
75/1023	627	211	18	733
75/1118	806	211	92	327
76/362	544	83	34	303
76/372	513	50	187	72
76/448	313	449	120	845
	534	425	64	543
76/595	416	475	27	1067
	443	77	51	828
76/595	390	313	25	900
76/618	346	239	35	1007
	319	181	68	1206
76/636	343	348	–	–
77/638	576	264	379	539
77/641	605	217	96	572
77/688	1238	117	423	311
77/699	922	231	67	411
	1512	161	144	539
	145	84	27	588
77/700	619	72	26	303
77/784	116	217	17	1253
77/3044	326	241	38	406
Mean	701	175	107	534

Later the copper levels (especially PCu) fall and so does iron. Bilirubin may continue to remain elevated for some time. The BU levels begin to rise relatively late and may only reach a peak several days or longer after the initial haemolysis. The course of the disease is frequently complicated by further haemolysis, as is evident in several of the clinical cases. If further haemolytic episodes occur, they entail repetition of the sequence of events as previously outlined so that high BCu and GOT levels may appear in a sheep already very anaemic and uraemic. Chronic cases are characterised mainly by severe anaemia and uraemia. BU levels are useful in the prognosis for eventual recovery. It is interesting to note that in the random flock samples, some cases consistent with the onset of haemolysis were found (for example, cases 74/965–18, 74/965–19, 74/988–1, 74/988–12, 76/618–12 and 76/618–13 in Table 2).

Urine analysis in acute cases consistently reveals the presence of protein, bilirubin and haemoglobin in moderate to large amounts.The reaction is generally acid and tests for ketones and glucose are usually negative. Iron and copper levels in urine approach those found in the plasma. Iron levels were between 15,8–54,8 μmol/ℓ while copper varied between 12,2–94,2 μmol/ℓ. All the values dropped gradually in an instance where the long term development of a chronic case was followed.

Levels of copper and iron in the kidneys and liver show important and constant changes. It is quite clear from the accompanying data that in nearly all cases the liver copper was above 300 μg/g wet mass (WM) approximately equivalent to 1000 μg/g dry mass (DM). Furthermore, no cases of kidney copper under 10 μg/g (approximately 30–40 μg/g DM) were found. The liver iron levels are generally elevated but the kidney iron levels are even higher. The latter levels are evidently a reflection of haemolysis and haemoglobin accumulation.

The means of copper for liver and kidney are 701 μg/g and 107 μg/g respectively, while that of kidney iron is 534 μg/g WM. These levels are grossly abnormal by any criteria[3 7 23 31 48], and the copper levels are considered highly suspicious if not diagnostic for chronic copper poisoning by most authorities[3 7 23 29–31 37 48 55].

In general the results of these liver and kidney analyses are similar to those quoted by Brown in earlier work by de Boom[10]. However, there are some considerable differences in the other data presented here compared to that reported previously by Brown[10]. Some possible reasons are to be discussed later in this paper.

Haematological studies have been undertaken by de Kock[21] and Brown and de Boom[12]. Our limited studies are in agreement with their work.

PREVENTION

The relatively low value of individual animals as well as the severity and rapidity of the clinical course of the disease has placed the emphasis of control measures in EI largely on prevention rather than treatment. It remains to be added that since most animals die despite all treatment and some animals can recover with no treatment at all, the alleged effect of various remedies must be evaluated with caution.

Because EI so closely resembles chronic copper poisoning in its outward manifestations at least, veterinarians of the Regional Veterinary Laboratory at Middelburg have over the past number of years been advising farmers experiencing losses from EI to treat the remaining sheep in the flock with ammonium or sodium molybdate. Initially this was done tentatively and experimentally but the results were so encouraging that it has latterly become standard practice. This preventative measure has also become known outside the enzootic area where outbreaks have occurred in sheep coming from these regions. In most cases further deaths and clinical cases stop dramatically. This is in sharp contrast to various other remedies and nostrums which are tried from time to time. While various supporters of individual home remedies claim good results, there is no general agreement on any one. Most farmers using molybdate report an immediate and marked drop in losses. Since they generally only begin dosing during an outbreak, poorer results could be expected. Sheep are already stressed, and dosing adds to the stress load. Where poor results have been reported from dosing of molybdate, the additional stress of dosing and handling generally seems to outweigh the probable benefit of reducing copper uptake and storage.

Reducing the liver copper status or reducing uptake should therefore preferably be done on a long term basis and not only when clinical cases occur. A fair number of farmers have in fact now taken to regular dosing of molybdate or supplying it in lick form. On these farms it is reported that EI has become much less of a problem. When taken individually or even in tens, favourable reports on any alleged remedy used under field conditions without proper controls must be interpreted with some caution. However, when the reports run into large numbers and favourable reports exceed unfavourable ones by better than nine to one, while in all other remedies the reverse is usually true, then some definite conclusions may be drawn.

Some amelioration may result from improving natural grazing and by feeding sheep during periods of drought. The elimination of various stress factors would be desirable in theory but often difficult if not impossible to carry out in practice. While improvement of the veld by grazing management certainly may be of value, especially by preventing severe nutritional stress in times of drought, this is not the whole answer to the problem. The disease continues despite the Stock Reduction Scheme and the very good seasons of 1974 to 1977 which brought about a marked improvement of veld generally. As a further example, at the Grootfontein Agricultural College, where sound veld management has been practiced for decades and where EI is generally regarded as being absent, older sheep slaughtered for consumption often have dangerously high levels of liver copper. In 171 samples from aged (full mouth) sheep, 41 were found to have liver copper levels of above 300 μg/g WM ($\pm$1000 μg/g DM). Our records show that EI does occur in sheep from the farm but usually in limited numbers unless experimental undernutrition has acted as a stress factor.

In theory one could avoid the problem by selling sheep above the six tooth stage. This would, however, be completely impractical since older ewes (which constitute the bulk of clinical cases) have been retained for their proven genetic worth and mothering ability. If EI is accepted as a form of chronic copper poisoning then British mutton breeds or their crosses would not be kept on affected farms unless copper accumulation in the liver is strictly controlled.

For these reasons the supplementation of molybdate remains the preventative method of choice at present. Most farmers use a 2 % solution of ammonium or sodium molybdate and dose 5 to 10 ml daily, every second day or weekly. This supplies between 100 and 200 mg per dose per sheep. Many farmers have developed their own variations on this basic recommendation. Other farmers are using various formulations of licks or supplementing molybdate in the drinking water. The use of these various methods of supplementation as well as the possibility of using other supplements like sulphur and zinc to reduce liver copper accumulation is currently under study.

RELATIONSHIP WITH OTHER DISEASES

Diseases like enterotoxaemia, ketosis and hypocalcaemia are precipitated by the same sort of stress factors which initiate outbreaks of EI. It is therefore evident that one or more of these diseases may well be present concurrently with EI and modify the course of the disease as well as its manifestations. This helps to explain at least some of the apparent aberrations which are seen in EI from time to time. Two or more disease conditions present at the same time would undoubtedly aggravate an already severe disease and probably result in a more rapid course, so that a full development of the characteristic lesions of EI would not be reached.

In addition, diseases like bacterial icterus, Rift Valley Fever, Wesselsbron disease, verminosis and geeldikkop or other forms of photosensitisation, all have some features in common with EI, notably icterus or anaemia. If these diseases act as non-specific triggers for EI, extreme confusion of the clinical and pathological picture could result.

Since Brown considered EI and geeldikkop as different manifestations of the same basic disease condition[8 9 10 11 13], the relationship between these two diseases must receive special consideration. At least some of the specific characteristics by which they are generally known and identified must be used in a comparison between the two diseases. The more non-specific characteristics which are common to many other diseases must carry less weight unless it is postulated that all the conditions involving these features are also somehow interrelated. Specific features common to both EI and geeldikkop are nearly all subject to qualification. Both diseases are characterised by a more or less severe icterus but only in EI is it characteristically a dirty brownish yellow. In both there is some degree of anaemia, in geeldikkop it is mild while in EI it is extremely severe. Both diseases may involve gastrointestinal stasis and splenomegaly but these lesions are not invariable or of vital significance. Certainly gastrointestinal stasis cannot be considered specific or diagnostic in either case.

Although photosensitisation of some degree (usually very severe) is a constant feature in geeldikkop, it is extremely rare in cases of uncomplicated EI. The most that is seen in the latter disease is a very mild reddening and crusting of the muzzle. Other lesions like conjunctivitis, keratitis and nasal or ocular discharges, though common in both diseases, are nonspecific and not related to photosensitisation as such.

Macroscopically the livers may appear somewhat similar but microscopically EI is characterised by a more centrilobular distribution of lesions, megalocytosis and a brownish pigment[10 20 21 49], while in geeldikkop almost diagnostic cholesterol-like clefts in hepatocytes and bile ducts are seen and lesions are more perilobular in distribution[62].

Nephrosis is seen in both diseases but the brownish-black pigmentation seen macroscopically in EI and the microscopic picture dominated by haemoglobin in the tubules, which is so characteristic of EI, is never seen in uncomplicated geeldikkop[21 49 62].

The differences are far more numerous. Many of the vital points have already been noted in certain apparent similarities. haemoglobinaemia, haemoglobinuria and methaemaglobinaemia (which gives blood its characteristic colour in EI) are all absent in geeldikkop while they are almost diagnostic in EI. High plasma copper levels may be seen in geeldikkop but never reach the same heights as in EI. It bears mentioning that moderately elevated plasma copper levels are a non-specific concomitant of many diseases of sheep[19]. Blood copper, as a reflection of red cell copper, is extremely high in the majority of cases of EI.

Liver copper is always elevated in cases of EI[8 10 26 27] (see also Table 3) but variable in geeldikkop[10 26 27].

Erasmus has reported high liver copper levels in only a small minority of geeldikkop cases[27]. Kidney copper levels are within normal limits in geeldikkop in most cases reported by Brown but definitely raised in others[10]. Bearing in mind that the two cases quoted having kidney copper levels over 20 μg/g had liver copper values of 708 μg/g and 1520 μg/g WM, it seems more than likely that these sheep could have been suffering not only from geeldikkop but also from chronic copper poisoning. The kidney copper values reported in the present paper are all high, these levels are consistent with chronic copper poisoning[3 5 7 23 29-31 35 37 43 44 48 55].

Liver iron values in geeldikkop reported by Brown[10] are generally normal and those of the kidney are normal or markedly high (these cases may again be related to chronic copper poisoning). The figures in this paper show generally normal to raised liver iron and generally excessive values in the kidneys. This is in agreement with de Boom's findings on EI quoted by Brown[10]. Similarly the levels of plasma iron in EI reported in this paper are generally higher than those for geeldikkop[10].

In addition to these striking differences it must also be remembered that while geeldikkop is most severe in lambs, EI affects older sheep almost exclusively. Geeldikkop first appears after rains while in EI rains generally end an outbreak. Stress factors which feature in the latter disease are not important in geeldikkop. Merinos are more susceptible to geeldikkop but resistant to EI while the reverse is true of Dorpers.

While these two diseases therefore do resemble each other in certain respects, on the most important diagnostic criteria of each there are vital distinctions. When both diseases are present concurrently in an animal considerable confusion can result. It has been observed earlier that geeldikkop, like any other stress factor, may act as a trigger to an outbreak of EI. In these circumstances it would be a herculean task to try to sort out which finding should be attributed to which disease. This could certainly give the impression that the two diseases are closely related.

It has been established by Gardiner that sheep with high liver copper levels are more prone to lupinosis and suffer more severely from its effects[28]. Although to date this has not yet been reported in cases of geeldikkop, there appears to be no good reason why the same should not be true in the latter disease. The generally high copper levels of normal sheep in the karoo, where both EI and geeldikkop are most prevalent, has been previously observed by Brown[8 10] and Erasmus[26 27] and is confirmed by unpublished data at the Regional Veterinary Laboratory at Middelburg. Thus it is more than likely that many sheep affected by geeldikkop will have copper levels higher than sheep raised elsewhere. Indeed, at least part of the severe icterus, haemolysis and nephrosis sometimes seen in geeldikkop may be due to high liver copper levels which can result in the liberation of copper into the blood stream in times of stress. Some of Brown's data points to this[8 9 10]. In addition, animals with toxic levels of liver copper show increased levels of enzyme activity in the plasma[7 55 56], which presumably is indicative of liver damage. These livers might well be more susceptible to the toxin which causes geeldikkop. Thus EI-prone sheep might be more susceptible to and suffer more severely from geeldikkop and the latter disease may act as a trigger for the former. In this sense only are the diseases related; they may act synergistically though they are aetiologically distinct.

COMPARISON WITH CHRONIC COPPER POISONING

Chronic copper poisoning in its various forms is today a very well documented entity. Only a relatively few references are given to prevent repetition. Since enzootic jaundice or the "Yellows" of Australia has been shown to be a form of chronic copper poisoning, it has been included with chronic copper poisoning in the discussion although heliotrope poisoning is sometimes referred to separately. These latter forms of chronic copper poisoning are important in this discussion since they occur in sheep on range grazing. The findings of this paper on EI are usually not mentioned specifically but should be kept in mind in this comparison.

When comparing chronic copper poisoning and EI, it soon becomes clear that the similarities far outnumber the differences which are of a minor nature. The symptomatology of both diseases is characterised by depression[3 16 20 21 23 24 30 37 43 48 54], anorexia[16 20 21 24 29 43 48], weakness[2 3 20 21 24 29 37 48], rapid respiration and pulse[2 20 21 37 48 54], and rapid loss of condition[20 24 48 54]. Nasal discharge[2 20] and gastrointestinal stasis[3 20 24 37 54] may be present, while in other cases diarrhoea[2 24 29 37 54] may be seen. The temperature is variable[2 16 20 21 37 48 54]. The most diagnostic signs are dark reddish-brown urine[2 3 10 14 16 20 21 24 29 30 37 43 48 54], anaemia[2 3 7 10 16 20 21], icterus[2 3 7 15 16 20 21 23 30 35-37 48 54], haemoglobinaemia[3 10 12 14 21 54 61] and methaemoglobinaemia[10 12 20 21 23 24 60]. The last three characteristics are probably responsible for the characteristic dirty yellow colour[2 14 21 24 29 37 54] of the mucous membranes.

Gross pathology in both diseases is dominated by the same characteristic signs described under symptomatology viz. anaemia, icterus, methaemoglobinaemia and haemoglobinuria. The general colour of the carcass is often a dirty greyish, brownish or greenish yellow[2 14 21 24 54] and darkens on exposure to air[21 48]. Livers are swollen, yellow and friable in acute case[2 3 20 21 29 30 35 37 48 54], but become more fibrotic in chronic cases[2 20 35 37]. Kidneys are strikingly pigmented in both diseases, usually being an almost pathognomonic dark brown to black colour[2 3 10 14 17 20 21 24 30 35 37 48 54]. Gastrointestinal stasis is common (especially in chronic cases) but not invariable in either disease[3 10 20 21 24 37 54]. Dark faeces or frank blood may be present in the intestinal tract[2 3 21 37 54]. The spleen is often enlarged and the substance is frequently dark and pulpy[2 20 21 30 35 37 48]. While lymph nodes are swollen in acute cases, they are atrophied and pigmented in chronic cases[2 20 21 37]. Skeletal musculature is dark[2 48] and oedema of the lungs, hydrothorax, hydropericard and ascites are often present[20 21 29 35 37 54], usually with a yellow-brown tinge. There is rapid decomposition of the carcass[2 21 24 54] and an unpleasant odour[2 54].

In both diseases the chief histopathological liver lesions are centrilobular degeneration or necrosis[2 14 21 35 37 49 50 54], pigmentation[2 21 35 49 54], swollen hepatocytes[21 35 49 50] and karyomegaly with some nuclei containing vacuoles or inclusion bodies[35 44 49 50] while megalocytosis is reported in both EI and heliotrope poisoning[14 15 21 49]. Both diseases are further characterised by haemoglobin casts in kidney tubules and brownish pigment granules[2 14 21 29 35 49 50 54].

The haematology of both diseases, besides anaemia, is characterised by "ghost" erythrocytes which do not stain well, anisocytosis, poikilocytosis, Jolly bodies, normoblasts and polychromasia[2 10 12 16 21 54].

High plasma copper levels are seen in both diseases,

especially transiently just prior to the haemolytic crisis[3 10 14 23 30 43 60], while red cell copper levels may rise tenfold or more for a longer period[3 14 23 43]. This finding is very clear in the present work, where a number of gross elevations of plasma copper are present while many more extremely high levels are found in the whole blood analyses, which are an index of red cell copper. Both diseases also feature increased plasma levels of GOT[7 55 56]. Haemoglobinaemia and methaemoglobinaemia has been chemically determined in both diseases[10 12 60]. In chronic copper poisoning liver copper levels are high, usually above 300 μg/g WM (or 1000 μg/g DM)[3 5 8 10 14 15 29 30 44 48] although in some cases lower values are found, probably due to the duration of the disease where leaching of copper takes place[37 48]. The same picture is evident in EI from the analyses presented in this paper as well as de Boom's figures quoted by Brown[10] and those of Erasmus[27] who found levels between 500–1528 μg/g WM. It is important to note that, while liver copper levels of normal sheep from elsewhere are usually below 200 μg/g WM[3 7 8 10 23 48], this does not hold true in the case of karoo sheep. Brown has reported liver values of between 183 and 349 μg/g (mean 251 μg/g WM) for his control sheep[10] while Erasmus has given normal values of between 20 and 1400 μg/g DM (mean 475 μg/g)[26]. Values for normal aged Grootfontein sheep mentioned previously have a mean of 227 μg/g WM (171 animals). Therefore, high copper levels in the liver are not diagnostic of chronic copper poisoning but are indicative of potential toxicity if liberation of some of this copper into the bloodstream should occur. The kidney copper levels are of far greater value in diagnosing toxicity[3 23 31 44 48 52 55 56]. Normal copper levels are very seldom above 10 μg/g WM[23 29 44 48 56], while levels above 20 μg/g WM should be considered toxic[37 44 48 52 55 56]. In the present work no values under 10 μg/g were obtained, and only 4 out of 31 were under 20 μg/g. These values must be considered highly significant. Liver and kidney iron values are also important. While normally kidney values are less than those of the liver, in EI the reverse is usually true. Some limited evidence of this is found in chronic copper poisoning[25 29 48]. Elevated kidney iron content is probably a reflection of haemolysis.

Both diseases are of sudden onset and are triggered by non-specific stress[3 11 14 21 31 36 50 55] and outbreaks of EI and chronic copper poisoning under range conditions may occur in droughts[11 14 24 54]. Again, under natural conditions, both diseases are more common in aged sheep[2 10 11 14 20 24 36 37 54], and British mutton breeds or their crosses are more susceptible than Merinos[3 17 24 30 54]. The form that both diseases take is related to the duration of survival after haemolysis[24 36 48 54] and the degree of haemolysis[10 20 24 30 35]. In both diseases, mild or chronic forms are described as well as the classical severe or acute form[3 16 20 24 30 36 37 54] and there may be several haemolytic episodes[10 20 29 30 35 36]. Perhaps the most convincing evidence that EI is a form of chronic copper poisoning is that they can both be prevented by dosing salts of molybdenum[31 50 52 55–57]. It is difficult to see how this fact can be explained on any other basis.

Compared to these similarities, the differences are trivial and are rather variations in degree than absolute distinctions. Nervous symptoms[48] and brain lesions[35 44] which are described in chronic copper poisoning have not been recorded in EI, but have also not been specifically sought and eliminated. The enzootic area in South Africa is in general unsuited to British mutton breeds and is subject to severe droughts. Therefore, the relatively resistant Merino or crossbreeds are usually the animals involved. They appear to accumulate copper more slowly and need greater stress (usually nutritional) to trigger the disease. Many cases in South Africa therefore develop less rapidly than elsewhere, though the severe and rapid course is well known. These factors explain why findings associated with chronic forms are relatively more common in EI. Emaciation, severe gastrointestinal stasis, severe anaemia and elevated BU levels are to be expected under these circumstances. It has already been noted that where farmers have both Merinos and Dorpers (derived from the Dorset Horn and the Blackhead Persian) it is the Dorper which is more inclined to develop EI which also runs a more acute course than in Merinos. Other diseases may be present concurrently with EI and the resulting syndromes may well have been the apparently aberrant forms of EI described in the past.

MECHANISM OF COPPER ACCUMULATION

If EI is accepted as a form of chronic copper poisoning, the mechanism of accumulation of liver copper which preceeds the haemolytic crisis must be explained. While detailed work on this aspect has awaited confirmation that the diseases are identical in other respects, some possibilities can be deduced from available information.

Careful investigation of the history of outbreaks revealed that the use of remedies, molluscicides and dips containing copper was not a constant enough feature to account for the source of copper. The epizootiology of EI is strongly indicative of a poisoning of a cumulative nature. This has led to studies of the chemical composition of both plants and soils in the enzootic region[10 11 13 39–41] and to an evaluation of the plant populations and soil types involved[58].

Brown[8 10 11 13] found high levels of selenium in plants and the soil from EI-prone farms. He also reported de Boom's findings of relatively high levels of copper in plants from farms where EI is regularly severe[10]. Copper levels between 6 and 32 μg/g DM were reported, and the means of eight common fodder species varied between 14 and 18 μg/g. In this report de Boom is, however, quoted as finding that there was no difference between copper levels in soils or rocks from farms where the disease was either severe, or unknown. Dolerite samples were regularly high in copper, containing 180 to 335 μg/g of the element. Other rock and soil types were far lower. Brown nevertheless, concluded that these levels were not high enough to cause toxicity and that copper was not involved in the pathogenesis of the disease[10].

Louw and coworkers[39–41] could not find evidence to support the postulate that geeldikkop and EI were the result of subclinical selenium intoxication. They found levels of between 0,05 and 0,89 μg/g DM in 77 different plant species, compared to Brown's figures of 3 to 71 μg/g. They ascribed the higher values reported by Brown to the codein complex method he had used, which they found less reliable than their D A B method[39]. In this report, selenium content is shown to be roughly correlated to soil pH. The authors point out that the soils of the more mountainous parts where EI is more common, generally have a lower pH than do

the soils of the plains where geeldikkop is more common. Thus lower selenium levels were found in apparently more EI-prone areas. A relationship was found between the incidence of EI and the ether extract content of the grazing.

Neethling[45] subsequently analysed tissues for selenium by neutron activation and confirmed that levels were elevated, although not as high as in Brown's original work. It should be mentioned here that selenium supplementation has been shown to increase both selenium and copper levels in the liver[4]. Therefore, marginally high selenium intake would in fact promote chronic copper poisoning.

In later publications[40 41] *Louw and coworkers* analysed plants collected in summer and winter in the western mountain karoo (where EI is very severe) and in some adjacent north western karoo veld types, where the disease is also present in some parts. This supported their contention that selenium levels were not high enough to cause toxicity. In the same publications, in addition to many other analyses, values for copper and molybdenum in common plants are given. The accompanying table (Table 4) shows a summary of their findings. Generally copper levels were higher in summer than in winter. This has been observed elsewhere[10 36]. Copper levels from the western mountain karoo were generally higher than those of the north western karoo. Many of the plants in both areas had copper levels over 10 μg/g DM, and few plants had molybdenum levels over 0,5 μg/g in either district, while only five plants in the north western karoo and none in the western mountain karoo had molybdenum levels over 1 μg/g. However, the authors came to the conclusion that copper and molybdenum levels were such that neither deficiency nor toxicity could be expected.

Table 4: COPPER AND MOLYBDENUM VALUES OF GRAZING IN μg/g DM*

Number of plant analyses	Western mountain karoo		North western karoo	
	Summer	Winter	Summer	Winter
Mean copper	10,19	7,25	8,53	7,52
Mean molybdenum	0,47	0,52	0,53	0,38
Lowest copper	6,65	4,15	4,02	2,86
Highest copper	15,18	10,12	13,92	20,01
Highest molybdenum	0,73	0,64	1,94	0,88
Mean Cu: M ratio	26,64	23,61	21,41	26,89
Lowest Cu: M ratio	7,8	3,6	5,0	5,4
Highest Cu: M ratio	69,0	29,6	67,6	95,3

*Extracted from Louw, Steenkamp and Steenkamp[40 41].

In neither these publications[40 41] nor that quoted by Brown[10] is the soil type or geology of the exact area of collection mentioned. This may considerably affect the chemical composition of plants growing in adjacent parts of the same farm[10 14 36]. To arrive at a reasonable estimate of the probable copper and molybdenum intakes of sheep grazing on any pasture, the relative nutritional dominance of various plant species would also have to be considered. This implies that those species supplying the most fodder in a given region are relatively far more important than the rest. In addition, the sheep is well known as a highly selective grazer and samples collected by hand are often significantly different to those obtained by oesophageal fistula[6 18 38].

For these reasons, a small investigation was carried out in two camps of a farm on which EI had just broken out. Samples were taken of apparently dominant bushes growing on doleritic ridges, sandy alluvial soil and areas betweeen these extremes. The tiny leaves and growing tips of each plant species were then carefully stripped by hand and analysed for copper content. The results appear in Table 5. It is evident that the copper levels in nearly all cases were above 10 μg/g DM. The mean for samples on doleritic ridges was 17 μg/g (n = 10), that of middle veld 12 μg/g (n = 3), and that of the sandy flats 11 μg/g (n = 7). Means for the two camps were very close. These figures in general agree with those of de Boom but are higher than those of Louw. This might be due to the method of collection as well as the choice of bushes and the site of sampling. Further investigation is required.

The pH of soils also might have a decided influence on copper uptake in the sheep, since low pH levels inhibit both selenium and molybdenum uptake in plants[14 17 36 39]. Both these elements affect copper metabolism. It is well known that molybdenum depresses liver copper accumulation[22 31 34 42 59] while selenium enhances it[4 51 52]. The values quoted here therefore certainly do not exclude the possibility of chronic copper poisoning being the essential factor of EI. In fact, they support the contention since the occurrence of clinical EI mainly in older sheep indicates a gradual low level buildup of copper in the liver.

Although Skinner[58] observed that EI was more common on farms dominated by shales and sandstone than dolerite, the findings of this work indicate that the reverse may be true. A further factor favouring doleritic soils in the aetiology of the disease, besides the high copper levels in the parent rock, is the generally lower pH of these soils. However, since many farms containing extensive doleritic instrusions are seemingly free of EI and vice versa, there may be other special factors involved, possibly occurring only in certain localities. For example, low molybdenum levels in certain acidic soils and high selenium levels in certain basic soils could be responsible for the apparent anomaly. In addition to molybdenum and selenium, other elements like sulphur, zinc, manganese, iron, calcium, phosphorus and cobalt may be involved in a complex imbalance[19 25 31 33 36 42 51 52 61], while a poison similar to heliotrope cannot be ruled out. In fact, there are histopathological similarities[15 49].

Skinner[58] found a correlation between the incidence of EI and overgrazing or poor veld management. This indicates either that the plants which become dominant under these conditions supply a diet which is conducive to copper accumulation or that on these farms the fodder reserve is so low that in droughts nutritional stress is more severe and more clinical cases appear. Both factors may be in operation.

Several authorities have noted the occurrence of chronic copper poisoning in sheep whose feed or pasture was not grossly high in copper[7 14 22 31 32 36 48 52 53]. There seems to be a general consensus of opinion and experience that when feed copper levels are greater than 10 μg/g and molybdenum levels are less than 1 μg/g DM the danger of chronic copper poisoning exists. EI is largely confined to the oldest sheep in a flock, which indicates a gradual copper accumulation and therefore relatively low copper levels in the herbage. Even disregarding other mineral interrelationships

Table 5: COPPER LEVELS IN μg/g (DRY MASS) IN VARIOUS BUSHES IN TWO CAMPS OF A FARM WHERE ENZOOTIC ICTERUS *HAD OCCURRED*

Plant (Camp 1)	Site	Cu	Plant (Camp 2)	Site	Cu
Galenia procumbens	V	2,5	*Eriocephalus spinescens*	V	13,2
Pentzia spinescens	V	14,4	*Pentzia spinescens*	V	18,7
Eriocephalus pauperrimus	M	13,9	*Nestlera humilis*	V	10,5
Pentzia spinescens	M	10,5	*Pteronia glauca*	V	10,9
Eriocephalus ericoides	M	12,5	*Osteospermum spinescens*	V	10,2
Eriocephalus pauperrimus	R	22,4	*Eriocephalus spinescens*	R	11,0
Pentzia spinescens	R	16,0	*Eriocephalus pauperrimus*	R	17,1
Pentzia pinnatisecta	R	23,9	*Eriocephalus pauperrimus*	R	16,4
Eriocephalus ericoides	R	15,7	*Helichrysum luciliodes*	R	25,4
Lebecka spinescens	R	7,1	*Pentzia spinescens*	R	20,2

R = Doleritic ridges M = Middle veld V = Vlei and alluvial soil

which may be involved and remembering that due weight should be given to relative nutritional dominance of plants and selective grazing habits of sheep, on this basis chronic copper poisoning in the area of EI is a decided possibility. From the conflicting reports on levels of copper and molybdenum necessary to produce chronic copper poisoning[61] it is quite clear that dogmatic assertions regarding toxic or safe levels for sheep are extremely hazardous.

At present it is known that chronic copper accumulation is occurring in sheep in the enzootic region although the mechanism of this accumulation has not been properly elucidated.

CONCLUSIONS

While much work remains to be done on several aspects of EI, even on the basis of present imperfect knowledge certain conclusions can be drawn with reasonable certainty:

1 There is no firm evidence that the uncomplicated forms of geeldikkop and EI are closely linked in their aetiology. The diseases apparently may act synergistically but are aetiologically distinct. The enzootic areas of both diseases largely overlap so that their concurrent appearance in the same animal is a definite possibility and probably explains their apparent clinical congruence.

2 There are no reasonable grounds for continuing to doubt that EI and chronic copper poisoning are essentially the same disease. Their symptomatology, pathology, haematology, chemical pathology, histopathology and epizootiology are so similar that any other interpretation must be rejected.

3 The mechanism of copper accumulation remains in some doubt and should be the subject of further investigation, since this might in the long term influence control measures.

4 Finally, dependable and practical methods of reducing copper accumulation in sheep to safe levels under extensive conditions in the enzootic region must be found.

ACKNOWLEDGEMENTS

The Director of Veterinary Services is thanked for the opportunity and facilities to carry out this study. The help and encouragement of Dr E.M. van Tonder of the Regional Veterinary Laboratory was of great assistance in the course of the invesigation. The technical work involved was undertaken by several staff members. Mr A.W. Terblanche and Mrs M Horak of the Regional Veterinary Laboratory and Mr H.J. Vorster of the Grootfontein Agricultural College deserve special thanks. Numerous farmers who helped by donating sheep, assisting in field investigations and by sharing their knowledge on various aspects of the disease made a considerable contribution to this work.

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ELECTRICAL STUNNING OF KARAKUL LAMBS

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ABSTRACT: Kühne, K.J.; Jenkins, W.L.; Kruger, J.M. **Electrical stunning of Karakul lambs.** *Journal of the South African Veterinary Association.* (1979) **50** No. 1 15 (En), Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

An apparatus designed to electrically stun Karakul lambs prior to slaughter was tested for efficiency using the cardiac pain reflex and electrocorticograms to assess unconsciousness. The electrical shock delivered by the apparatus was found to produce unconsciousness, as opposed to curarisation, of sufficient duration to allow for subsequent slaughter.

INTRODUCTION

This study arose out of a request by Swakara, the S.W.A. Karakul Board, to evaluate the efficiency of an apparatus designed to stun Karakul lambs immediately prior to slaughter. Electrical stunning of animals prior to slaughter is widespread, and the accepted requirements of stunning apparatus have been reviewed by Warrington[7]. Because of the subjective nature of pain, it is necessary to utilize reflex indicators of pain perception in animal experimentation, and for an investigation of this sort in particular, it is in addition required that the reflex be functional independently of the motor system, in order to avoid the danger of regarding an animal which is merely "electrically curarised" as being unconscious. For this reason the cardiac pain reflex as described by Croft[2] was used. An attempt was also made to correlate the state of consciousness of the animal with the electrocorticogram.

EXPERIMENTAL PROCEDURE

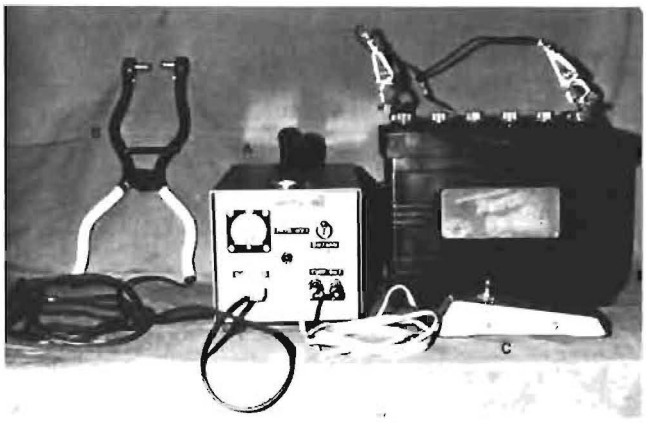

Fig. 1. The apparatus assembled for use, showing the power unit (A) connected to the battery, the electrodes (B) and foot switch (c).

Apparatus (Fig. 1)

The apparatus consists basically of a transistorised DC-AC converter with the following specifications:

Input: 12 volt DC

Output: (relative to 12v DC input)

(a) Voltage	110 volt AC
(b) Wave form	Square
(c) Frequency	50Hz.
(d) Power rating	120 watts

(e) Current capability in excess of 1 Amp

The circuit includes a time-switch which maintains the current for 5 seconds. The apparatus can be activated by means of either a hand or a foot switch. Current is applied to the animal by means of metal electrodes mounted in a pair of insulated spring-loaded calipers.

Experimental animals

The animals used were 6 Merino lambs approximately 2 weeks old and 12 Karakul lambs 12–48 hours old. As it was found that animals recovered fully from the experimental treatment, some of the lambs were used repeatedly.

Experimental method

Following induction of general anaesthesia using fluothane applied with a mask, metal electrodes (stainless steel gramaphone needles) with a polyethylene insulation were placed bilaterally at a point on the temporal line approximately midway between the lateral canthus of the eye and the base of the external ear. The electrodes were placed so that the point penetrated the skull and just penetrated the outer layer of the cortex. Positioning of the electrodes was confirmed by radiographs (Fig. 2).

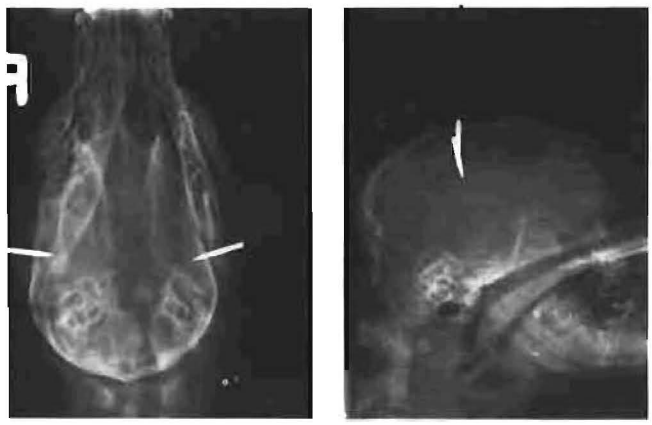

Fig. 2. Radiographs showing the EcoG electrodes in situ.

After allowing the animals to recover from the anaesthetic, they were restrained either on an insulated table or in a specially designed sling. The animal was then connected to an electrocardiograph using the standard limb leads and to an electroencephalograph via the implanted cranial electrodes.

Recordings were taken at rest and following application of a pain stimulus (nose-prick).

An electrical current was applied to the wet skin with the tong electrode placed just adjacent to the implanted

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electrodes. In some cases the tong electrodes were placed in the external auditory meatus. Following current application, the pain stimulus was again applied. The animals were either allowed to recover or sacrificed by severence of the carotid arteries immediately following the shock. For purposes of comparison, identical recordings were obtained from animals sacrificed without prior electrical stunning and from animals anaesthetised with thiopentone sodium.

Table 1: VARIATION IN RESISTANCE (Ohms) BETWEEN OPPOSITE SIDES OF THE HEAD IN KARAKUL LAMBS UNDER DIFFERENT CIRCUMSTANCES.

Sheep No.	Dry		Wet		Electrode gel	
	in ear	in front	in ear	in front	in ear	in front
1	10,000	17,200	11,500	3,500	3,500	3,500
2	11,000	120,000	11,000	5,500	6,500	4,800
3	11,800	55,000	18,000	6,000	5,000	6,000
4	22,000	40,000	22,000	9,000	19,000	10,000
5	20,000	11,000	20,000	5,000	6,000	13,500
6	30,000	200,000	28,000	9,000	22,000	4,800

RESULTS

Electrical resistance across the skull (Table 1)

Tests performed on 6 lambs prior to stunning showed that there was a significant difference in the electrical resistance across the skull between the two chosen sites for current application, provided that the site was either wet or electrode jelly was applied. The use of the apparatus on dry skin was unsatisfactory. There was a considerable variation in resistance between different animals of the same breed.

Current output by the apparatus

Due to the variation in the resistance described, a variation in current flow was found to occur. The voltage varied between 90 and 130 volts. The usual pattern of current flow was an initial flow of 120–180 mAmps increasing to up to 350 mAmps over the 5-second period. A current flow of under 60 mAmps was ineffective in inducing a satisfactory shock. The current cycle frequency was tested at 50 Hz.

Pain response

The change in heart rate in response to a nose-prick was evaluated (Table 2 and Fig 3). In 12 of 15 lambs an increase in heart rate (average 17,5 %) was obtained. The response was abolished by barbiturate anaesthesia and by an adequate electrical shock. Following electrical shock this pain reflex response was absent for at least 60 seconds (in 1 case 150 seconds) but had reappeared by 90 seconds.

Table 2: THE CARDIAC PAIN REFLEX IN LAMBS

Number	Breed	Heart Rate Normal	Heart Rate After Pain Stimulus	Duration of change	% increase
1	Karakul	180	210	4 sec	16
3	Karakul	160	160	–	–
4	Karakul	140	180	6 sec	28
5	Karakul	140	160	3 sec	14
6	Karakul	140	160	4 sec	14
9	Karakul	160	180	4 sec	12
17	Karakul	160	160	–	–
21	karakul	120	140	4 sec	16
22	Karakul	100	120	2 sec	20
1	Merino	180	220	3 sec	22
2	Merino	160	180	4 sec	12
3	Merino	140	160	3 sec	14
4	Merino	140	180	3 sec	28
5	Merino	160	160	–	–
6	Merino	140	160	4 sec	14

Physical effects of electrical shock

Following an adequate shock the typical sequence of events of an electropletic fit were seen:

1. Initial momentary flexion of the hind legs, followed by violent extension; the forelegs were stiff and extended at this stage and the head was flexed strongly backwards. Respiration was arrested for 0 to 10 seconds.
2. Rigidity gradually passed off and weak clonic contractions of all limbs occurred for 10 to 30 seconds.
3. Respiration restarted and the animal became flaccid for 30–60 seconds.
4. Muscular tone normal, but the animal lay motionless for periods up to 5 minutes. It was found that at this stage the animal was able to remain standing if placed in a standing position; it tended to remain in whatever position it was placed in.

Electrocorticogram (Tables 3,4 and Figs 4, 5, 6)

The normal awake animals showed a characteristic low amplitude wave pattern with a cycle frequency of 5–8 cycles per second. Following barbiturate anaesthesia the amplitude increased markedly and the cycle frequency changed to 2–3 cycles/second. Following electrical shock the electrocorticogram signal was unstable for a variable period and the first readable registrations

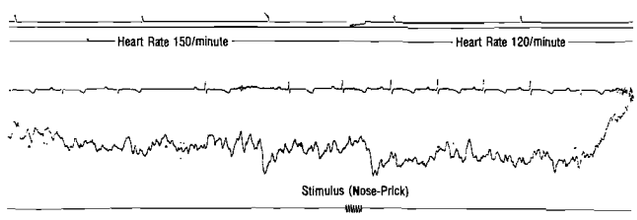

Fig. 3 The cardiac pain reflex.

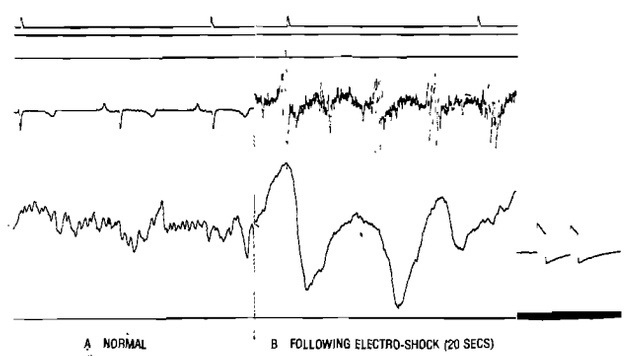

Fig. 4 Electrocortigrams from a lamb following electro shock.

Table 3: EFFECT OF SACRIFICE, WITH AND WITHOUT PRIOR ELECTRO-SHOCK ON THE ELECTROCORTICOGRAM OF LAMBS.

Number	Breed	Normal frequency cycles/sec	Treatment	Electrocorticogram
15	Karakul	5	Sacrifice	Flat after 25 secs
16	Merino	4 – 6	Sacrifice	Flat after 25 secs
17	Karakul	4 – 6	120 mA + Sacrifice	Flat after 64 secs
18	Karakul	3 – 4	105 mA + Sacrifice	Flat after 30 secs
19	Karakul	1 – 2	135 mA + Sacrifice	Flat after 25 secs
20	Karakul	1 – 2	180 mA + Sacrifice	Flat after 28 secs
21	Karakul	5 – 7	195 mA + Sacrifice	Flat after 25 secs
22	Karakul	4 – 6	240 mA + Sacrifice	Flat after 43 secs
25	Merino	7	150 mA + Sacrifice	Flat after 45 secs

Table 4: THE EFFECTS OF ELECTRO-SHOCK AND BARBITURATE ANAESTHESIA ON THE ELECTROCORTICOGRAM WAVE FREQUENCY AND AMPLITUDE IN LAMBS.

Number	Breed	Normal frequency cycles/sec	Treatment	Post-treatment frequency	Amplitude
1	Karakul	5 – 7	180 mA	1 – 2	↑
2	Karakul	5 – 6	97,5 mA	1 – 2	↑
3	Karakul	6 – 7	165 mA	1 – 2	↑
4	Karakul	6 – 8	180 mA	2	↑
5	Karakul	5 – 7	162 mA	1 – 2	↑
6	Karakul	6 – 8	225 mA	1	↑
7	Karakul	7 – 9	150 mA	2 – 3	↑
9	Karakul	5 – 6	240 mA	3	↑
11	Karakul	6	285 mA	2	↑
12	Karakul	5 – 7	300 mA	3 – 4	↑
14	Karakul	6	180 mA	2	↑
26	Karakul	5 – 6	Pentothal	2	↑
28	Karakul	5	Pentothal	3	↑
29	Karakul	5 – 6	Pentothal	2	↑
30	Karakul	7	Pentothal	3	↑
27	Merino	5 – 6	Pentothal	1 – 3	↑

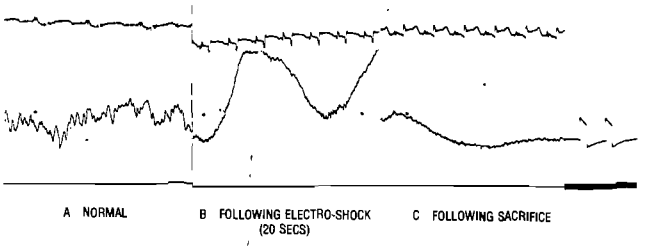

Fig. 5 Electrocortigrams from a lamb following electro shock and sacrifice.

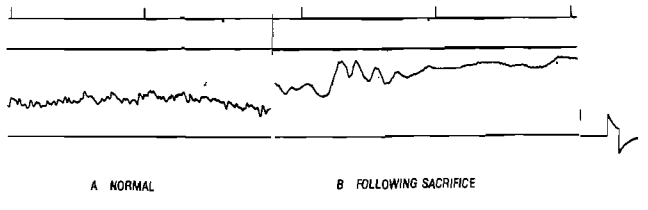

Fig. 6 Electrocortigrams from a lamb following sacrifice.

showed a very high amplitude wave pattern with a cycle frequency of 1–3 cycles/second. These waves were found to be present long after the animal had regained consciousness and were therefore not considered to be a reliable indicator of the duration of unconsciousness. Where sacrifice followed the electrical shock the electrocorticogram flattened within 40 seconds of the shock. Where animals were sacrificed without prior shock brain activity ceased within 25 seconds.

DISCUSSION

In outlining the experimental procedure three major questions were posed: (1) Does electrical stunning render the animal unconscious? (2) If so, what is the duration of unconsciousness? (3) Is electrical stunning a humane procedure, i.e. is it a painful experience for the animal?

Croft[3 4] considered that a fit with extensor tonus was an essential reaction to stimulation if unconsciousness was to be achieved. Secondly, her experiments showed that after electroplexy there were three distinct stages of recovery before normal consciousness returned:

a complete unconsciousness, with no reflexes present;
b consciousness of pain, as shown by a positive cardiac pain reflex, with paralysis of voluntary muscles – "electrical curarisation";
c return of reflexes involving voluntary muscle but absence of movement unless forcibly disturbed.

In our experiments the typical electroplectic fit always followed an adequate shock and with it there was a disappearance of the pain reflex (also abolished by barbiturate anaesthesia); this was accepted as an indication that a state of unconsciousness had been achieved. The validity of these parameters had been confirmed by Breazile *et al*[1].

It was found that, provided the skin was wet, the apparatus is capable of administering an electrical shock which renders the animal unconscious and insensitive to pain for at least 60 seconds. This was more than adequate time to allow for slaughter by severance of the carotids and destruction of the upper spinal cord. Croft[3] showed that a minimum amperage of 25–30 mAmps, a

minimum frequency of 50 Hz and a minimum time of 2 seconds were required to deliver an effective shock. Increasing the amperage from 30 to 80 mAmps, made little difference and increasing the cycle frequency above 100 Hz resulted in pain when the current was switched on. It was felt that the 5 second current application of the apparatus was an advantage in view of the changes which occurred in the current flow. That these minimum values are adequate for most domestic species which are slaughtered in this manner is confirmed by their application to commercial stunning apparatuses[7] and by the fact that the apparatus under test delivered an effective shock to an adult Merino sheep.

That the stunning process itself is painless has been demonstrated experimentally by Breazile *et al*[1] and is borne out by the reports of humans who have been subjected to electro-convulsant therapy. Associated with this therapy there is also a state of retrograde amnesia which in itself indicates a profound alteration in consciousness.

The interpretation of the electrocorticograms was made difficult by the instability of the signal following application of the shock. The initial flattening of the ECoG described by Croft[3 4] was not clearly seen but may well have been present during the time immediately following the shock when the recordings were not registering. Since the later changes in the wave form closely resembled those described by her, there is no reason to suspect that our results were any different. The results of ECoG activity following barbiturate anaesthesia and sacrifice closely followed that described by Martin *et al*[5] and Rössner and Westhues[6].

From these results it is concluded that the unconsciousness produced by the electrical shock delivered by the apparatus tested was of sufficient depth and duration to allow subsequent humane slaughter.

ACKNOWLEDGEMENTS

This work was funded by a grant from SWAKARA.

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BOOK REVIEW **BOEKRESENSIE**

PATHOGENESIS OF CYATHOSTOME *(Trichonema)* INFECTIONS IN THE HORSE : A REVIEW

C.P. OGBOURNE
Commonwealth Institute of Helminthology
Miscellaneous Publication No. 5.
CAB, Farnham House, Farnham Royal, Slough, England
SL2 3BN 1978 pp. II + 25 Figs (24(4 colour) Tabs 0 Publ. Price £4,00

Much of the fundamental work Dr. Ogbourne has reviewed has not been available for various reasons. It was either written in Russian or had a very limited circulation eg. a thesis in Edinburgh University (Mathieson, 1964).

This excellent review is divided into: introduction, pathogenesis of mature worms, pathogenesis of larval stages, clinical signs and haematology. Horses become heavily infested in summer and autumn but only young horses 1–3 years show clinical signs in the following spring. The most important signs are chronic diarrhoea, emaciation, cachexia, progressive weakness, recumbency and death.

Intermittent colic is not a consistent feature. At necropsy, the mucous membrane of the caecum and proximal part of the ventral colon may show 20 to as many as 50 nodules/cm^2, a catarrhal, haemorrhagic, or even fibrinous typhlitis and colitis. The pathogenic stages are 4th stage larvae or early 5th stages emerging from the gut wall.

Important diagnostic features are the rise in β globulins to as high as 7,0–8,0 g/100 ml of serum and either negative or low worm egg counts i.e. not exceeding 200 epg.

Fenbendazole (Panacur) at 30 mg/kg kills 90 % or more larvae in the gut wall and outbreaks can be prevented by dosing horses in winter.

I recommend this publication for all veterinarians interested in horses.

R.K.R.

DISLOCATION OF THE ELBOW AND ITS SOCIAL CONSEQUENCES FOR AN AFRICAN ELEPHANT

A.J. HALL-MARTIN* and H.P.A. DE BOOM†

ABSTRACT: Hall-Martin A.J.; de Boom H.P.A. **Dislocation of the elbow and its social consequences for an African elephant.** *Journal of the South African Veterinary Association (1979)* **50** No. 1 19 (En) Mountain Zebra National Park, P/Bag 66, 5880 Cradock, Republic of South Africa.

A dislocated elbow of a male elephant calf *(Loxodonta africana)* in the Addo Elephant National Park resulted in it being harassed by other elephants and consequently leaving its maternal herd at the age of 6 years, rather than the more usual age of 9–10 years. In the absence of large predators the elephant had survived for 9 years and had adapted its locomotion and intraspecific behaviour to its injury. The humeral joint of the affected forelimb was carried in partial extension while the cubital and carpal joints were permanently in partial flexion. Substantial secondary osteoarthritic changes, extensive fusion and compensatory remodelling of the humerus, radius and ulna had taken place together with compensatory development in the musculature. The planes of articulation of the limb bones had also been considerably rotated inward.

INTRODUCTION

A crippled young bull elephant *Loxodonta africana* (Blumenbach) was observed over a period of 9 years in the Addo Elephant National Park, South Africa. The animal carried its left forelimb with the humeral (shoulder) joint in partial extension creating the appearance of 'dropped elbow' (radial paralysis). The cubital joint (elbow) was permanently in partial flexion and so was the carpal joint (wrist). The olecranon projected laterally, causing a very prominent lateral bulge at the elbow joint, the triceps line also being displaced in as much as it was rotated lateralwards at its distal extremity. In this way a broad lateral ridge was created. These features are all shown in Fig. 1.

Fig. 1 A 14-year-old bull elephant showing the attitude of the dislocated elbow joint during locomotion.

HISTORY, SOCIAL AND LOCOMOTORY BEHAVIOUR

This animal was first reported in 1969 by a Park Ranger who estimated its age at the time as 5 years. It was still a member of a family group and its relationship with other members was normal. Even at that stage, however, older males were intolerant of the crippled animal, especially at water troughs (there being no natural perennial water in the Park). He was regularly harassed, threatened, prodded by tusks and pushed away from the water. On one occasion he was lifted bodily off the ground by a large bull.

Soon he was seldom seen with his family group and from the age of about 6 years was always alone. There was no danger of the young elephant being taken by large predators as they are extinct in the area. When he encountered other elephants at a waterhole he would turn away and wait at a safe distance until they had left before venturing near. Likewise, if other elephants approached while he was drinking he would immediately hobble away as fast as he could. The injured elephant moved with an awkward shuffle supporting his weight on the right forelimb while the injured leg was dragged and swung forwards. The sound leg was then quickly kicked forward while the weight was supported on the left leg with the trunk usually touching the ground as in Fig. 1.

When approached on the ground and on one occasion by a helicopter, the elephant always swung away to the left so as to present his right side to the source of disturbance. On the 23rd April 1977 the animal was shot. Examination showed that he was in good condition, indicating adequate nutrition. Because of the constantly flexed position of the carpal joint only the dorsal (anterior) edge of the foot was in contact with the ground.This resulted in the nails (hooves) of the first, second and fifth toes being grossly elongated, the first two growing forwards 9–10 cm and the fifth one growing lateralwards 8 cm. The fissured layers of cornified skin on the sole of the foot, which did not come into contact with the ground, were greatly thickened and in places had formed rough ridges protruding 3–4 cm from the general surface level. There had been much compensatory development in the musculature of the upper part of the right forelimb and shoulder regions, and a distinct right lateral curvature of the spine in the anterior thoracic region was evident. Using the criteria of Laws[2] the age of the animal was estimated at 14 years, thus confirming the estimated age at the time the injury was first reported.

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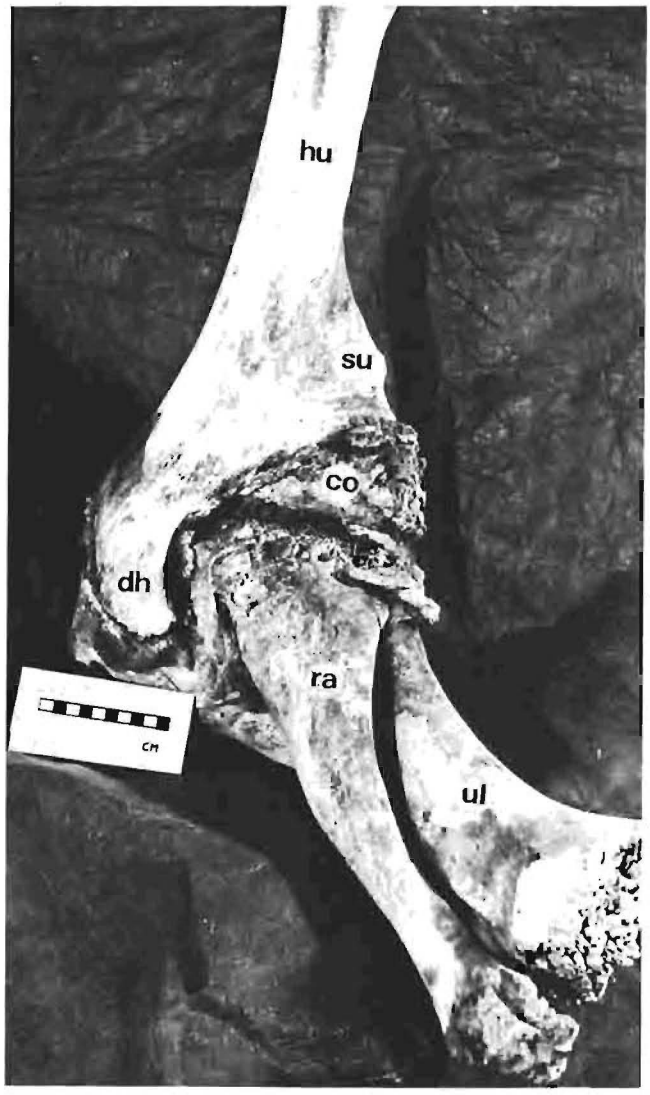

Fig. 2a Frontal view of the humerus (hu), radius (ra) and ulna (ul) of the injured elephant showing remodelling of the bones with the supracondylar ridge (su) of the humerus reduced, the distal end of the humerus (dh) growing around the head of the ulna and the replacement of the epicondyles (co) by secondary bone growth.

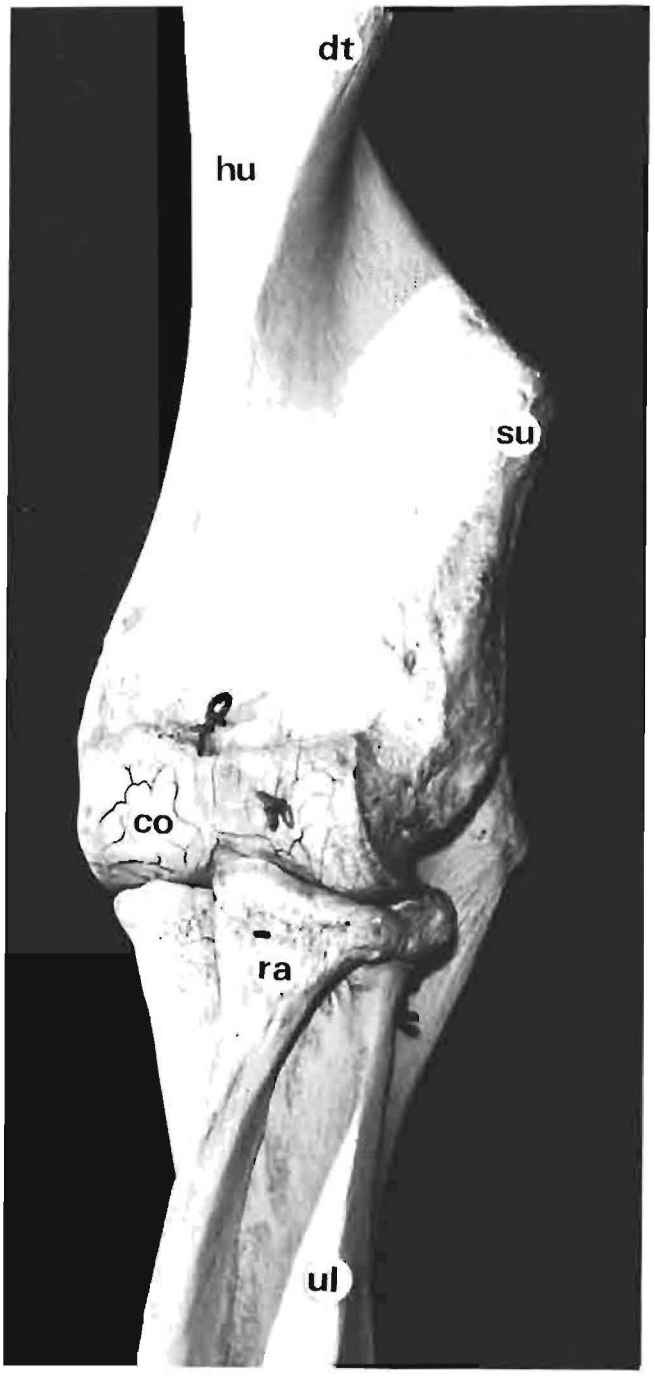

Fig. 2b Frontal view of the elbow joint of a normal elephant showing marked deltoid tuberosity (dt) and supracondylar ridge (su), smooth articulating surfaces of the epicondyles (co), and marked angularities on the surface of all the bones.

THE LESION

The lesion (Fig 2a and 3a, compared to normal bones depicted in Fig 2b and 3b) consisted of a dislocated cubital joint in which the vertical axis of the forearm had been rotated lateralwards. Secondly osteoarthritic changes at the joint and compensatory remodelling of the distal end of the humerus and proximal end of the ulna and radius had taken place. Disuse had resulted, furthermore, in atrophy of the normal ridges and tuberosities of the bones which had become either entirely obliterated or severly repressed. So, for instance, the deltoid tuberosity (dt) was represented by a fairly flat; rough area. The lateral epicondyloid crest or supracondylar ridge (su) was greatly reduced and the musculospiral groove on the humerus was barely discernible. Furthermore, the humeral condyle (co) had been eroded over that (lateral) part still in contact with the radius and ulna, whereas medially it had enlarged, projecting as a thick plate of bone distalward, making contact with the medio-proximal part of the ulna (dh in Fig. 2a). Secondary bone formation (sb in Fig. 3a) had caused extensive fusion with the medio-proximal end of the ulna. Extensive osteophytic growth had taken place at the edges of the contact area between the humeral condyle and the ulna and radius. As a result of the injury the distal end of the humeral shaft had become much wider medio-laterally and the normal caudally situated wide groove between medial and lateral epicondyles had been filled out with bone, thus presenting a large flat caudal surface. The radius was twisted over the ulna as would occur in full pronation, which is the normal position in the elephant (Fig. 2b) but the proximal end of the radius was widened mediolaterally and craniocaudally to almost twice the normal dimension. A similar widening of the proximal end of the ulna had also occurred, with filling out of the normally occurring shallow fossa present on the medial end of the ulna.

Fig. 3a Lateral view of the humerus (hu), radius (ra) and ulna (ul) of the injured elephant showing massive secondary bone growth (sb) at point of contact between the bones replacing the epicondyles, the lateral projection of the olecranon process (ol), and the reduced deltoid tuberosity (dt) and supracondylar ridge (su).

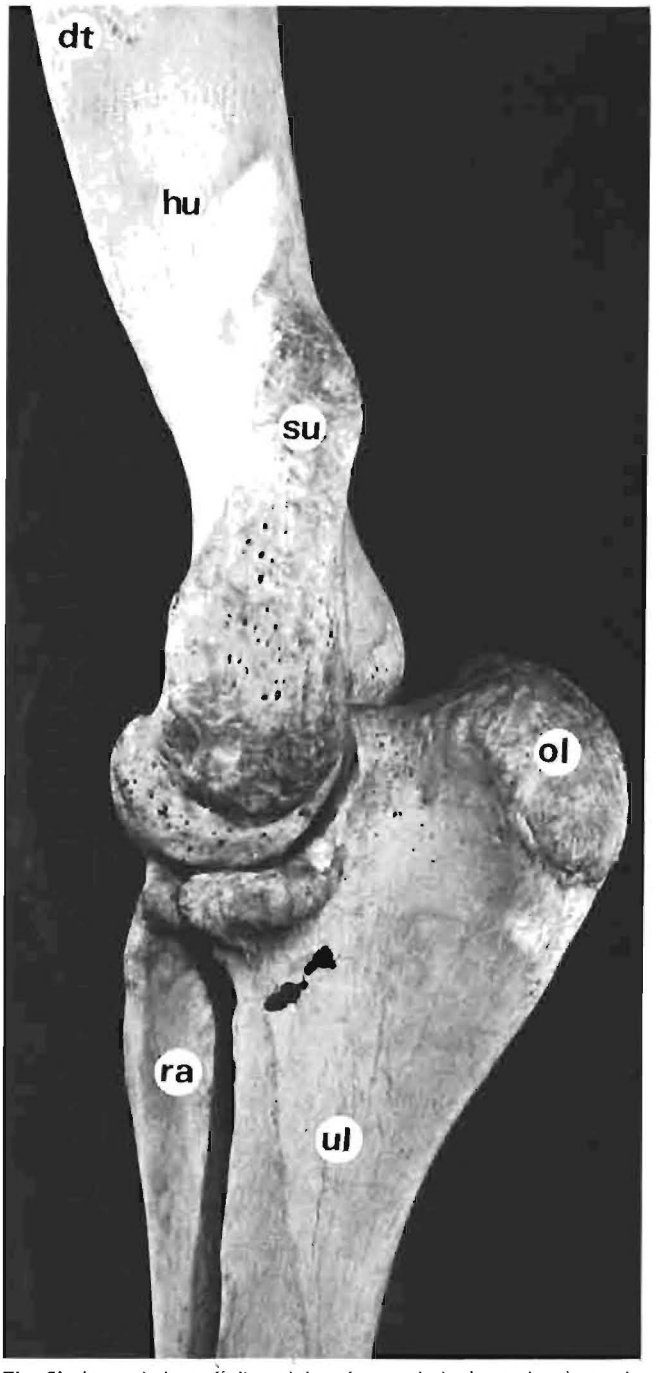

Fig. 3b Lateral view of elbow joint of normal elephant showing articulation of the epicondyles and caudal projection of the olecranon process (ol).

The olecranon (ol in Fig 3a and 3b) projected lateralwards instead of caudally. The plane of articulation distally (at the carpus) between radius and ulna had been rotated inward (pronation) through approximately 45°, whereas at the elbow joint the rotation had been virtually 90°. The proximal ends of radius and ulna were fused over an extensive area, and the medial part of the humeral condyle was fused with the proximal end of the ulna. A slight movement at this joint noted in the macerated specimen (increased to some degree by handling), was due to incomplete ossification of the epiphyseal line of the humeral condyle.

PATHOGENESIS

The kinetic pathogenesis of the lesion may be reconstructed as follows. During fairly fast forward progression, or as the result of a sudden fierce shove by another elephant, the subject stumbled on its left forelimb, the foot being held firmly under the body by some impediment. Continued movement diagonally forward and to the left of the body forced the elbow to rotate outwards and the forearm to assume a semi-horizontal position almost at right angles to the sagittal plane. Further movement of the body mass in this direction forced the humeral condyle to break its contact with the radio-ulnar articular surface, the humerus being levered out of the elbow joint, which formed the pivot for this action.

DISCUSSION

A similar injury to an African elephant has been documented by Gainer[1]. In the latter case it was a dislocation of the femur and tibia-fibula articulation. As pointed out by Gainer[1] the gravi-portal limb arrangement of the elephant makes the limbs less able to accommodate bending movements. The cubital joint of

the elephant also does not form such a snug-fitting ginglymus (hinge-joint) as it does in most quadrupeds. Additionally, the relatively long humerus and the body mass of the elephant could make it more susceptible to this type of injury than most other animals. Nevertheless, such injuries appear to be rare.

Our own (unpublished) observations on the behaviour of the Addo elephants indicate that the young bulls start becoming independant of the family unit at 9–10 years of age. The reports on the study animal, however, indicate that he left his family much earlier and led a solitary existence atypical of normal elephant behaviour. This was apparently a direct consequence of his injury, making him unable to react normally to the social and aggressive contact of other bulls.

ACKNOWLEDGEMENTS

We thank Mr P van Straaten and Mr K Goliath for their reports on the history of the injured animal, Professor J D Skinner for facilities at the Zoology Department, University of Pretoria and Mr W. Massyn for technical assistance.

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BOOK REVIEW — BOEKRESENSIE

ATLAS OF TOPOGRAPHICAL SURGICAL ANATOMY OF THE DOG

K. AMMAN, E. SEIFERLE, G. PELLONI
Paul Parey, Berlin, 1978. pp. XVI + 61 Figs 95 (Colour) Price DM 180.

The colour illustrations in this atlas represent biomedical illustrative art at its best. Indeed, they can be compared only with the best that have appeared in the fields of human and veterinary anatomy. The illustrations are superb, meticulously executed to the finest anatomical detail, and they bear testimony to the enthusiasm and devotion of the artist to her life-work. When viewing these artistic drawings it is becoming to recall the famous and immortal words of the poet: "A thing of beauty if a joy forever". In its form and character the book maintains the best traditions of anatomy and surgery and is entitled to a place alongside the finest of those emanating from the German and Swiss schools.

During recent years it has become customary in those schools to involve anatomists and clinicians in writing books of an applied nature – a laudible and deserving idea. The authors of this atlas are well-known experts in their respective fields and need no introduction.

Their combined efforts have resulted in a work on the surgical anatomy of the dog. The standard surgical approaches to the head, trunk and limbs are depicted and the topographical relationships of the fascia, muscles, ligaments, blood vessels and nerves is illustrated in the operative field: in a number of approaches even at different levels. The anatomical details are portrayed faithfully, in fact, the atlas could have been titled just as well as an atlas of regional anatomy. Surgical procedures are not described. The illustrations are labelled strictly according to the official international veterinary anatomical nomenclature which makes the atlas accessible to any language group. The captions are in German, English, French, Italian and Spanish. In some instances the English translation of the Germany captions is clumsy and in Fig. 22 it is totally wrong. This does not however, detract from the excellence of the book.

The typographical presentation, the excellent quality of the paper, and the outstanding reproduction of the colour drawings are prominent features of the book. As an aid in the study of the applied anatomy and operative surgery of the dog, this atlas will be invaluable to students and teachers. It should be available in every students' library and it should decorate the bookshelf of every small animal practitioner. The reviewer recommends this atlas without reservation.

J.M.W. le R

THE SEASONAL INCIDENCE OF HELMINTH PARASITES OF CATTLE IN THE NORTHERN TRANSVAAL BUSHVELD

J. SCHRÖDER

ABSTRACT: Schröder, J. **The seasonal incidence of helminth parasites of cattle in the Northern Transvaal Bushveld.** *Journal of the South African Veterinary Association* (1979) **50** No. 1 23 (En) MSD Research Centre, Private Bag 3, Halfway House 1685, Republic of South Africa.

During a period of 14 months two tracer calves per month were slaughtered after one month's exposure to natural helminth infestation on a farm in the Northern Transvaal Bushveld. Total parasitic helminth burdens, which were never large, were determined in order to define their seasonal incidence. The gastro-intestinal parasites that were recovered were *Bunostomum phlebotomum, Cooperia pectinata, Cooperia punctata, Haemonchus placei, Oesophagostomum radiatum* and *Trichostongylus* spp. Of these *Cooperia* spp. were most prevalent, and the highest mean monthly burden attained was 1250 in November, 1976. Worm burdens were apparently positively correlated with rainfall, which during the survey period was lower than the average for the particular region. In addition to the more common gastro-intestinal nematodes, *Thelazia rhodesii* and *Parafilaria bovicola* were also encountered. Various techniques employed in conducting helminth surveys are discussed.

INTRODUCTION

The seasonal incidence and epizootiology of helminth infestations in sheep in South Africa have been extensively investigated and are well-documented[4 17 21 25 27]. There is, however, a deficiency in our knowledge regarding the incidence, epizootiology and economic significance of helminth infestations of cattle in this country. Reinecke[19] did a field study on nematode parasites of cattle in the vicinity of Vryburg (North-Western Cape), Hobbs[10] undertook a survey of the seasonal incidence of cattle nematode parasites in the Natal coastal area, and Horak & Louw[11] slaughtered tracer calves over a period of 16 months in an experiment conducted at this laboratory.

The present survey was conducted on a cattle ranch in the Northern Transvaal Bushveld to determine the species of helminths prevalent in cattle in that area, the numbers they attain and the seasonal fluctuation in the availability of infective larvae on pasture. This was done to provide a basis for the planning of a cattle drenching programme.

MATERIALS AND METHODS

Trial location

The ranch is located in the Mixed Bushveld[1] of the Waterberg District (approximately 24°05′ S, 27°35′ E, Alt. 990–1035 m). The grazing is natural and unimproved, and consists of dense *Acacia nigrescens* ("knoppiesdoring") bush (trees and shrubs), interspersed with *Grewia* spp. ("rosyntjiebos"). *Dicrostachys cinerea* ("sekelbos"), *Combretum apiculatum* ("rooibos") and *Terminalia sericea* (Transvaal silver leaf) occur in lesser numbers, and a few solitary *Combretum imberbe* (leadwood) are also encountered in the paddocks grazed by the trial animals. The soil is sandy and the most prevalent grasses are *Digitaria* spp., *Panicum maximum, Eragrostis rigidior* and *Aristida* spp. There is no natural open water source and drinking water is supplied in troughs filled from boreholes. Rainfall was recorded by the farm manager for the duration of the experiment. The experimental animals grazed three paddocks with an approximate area of 910 ha out of a total of ca 2 350 ha.

Experimental animals

Two steer calves, to act as tracers of helminth infestation, were selected on the third Wednesday of each month, identified with numbered eartags and drenched with a broad-spectrum benzimidazole anthelmintic. They were drenched again 14 days later, and again on the third Wednesday of the following month before being exposed to infestation for one month prior to slaughter. These calves were born during the calving seasons of November-December 1974 and June-August 1975, and were all between the ages of nine and 19 months when they were slaughtered. During a period of 14 months from October 1975 until November 1976 two of these calves were slaughtered for helminth recovery every month, after having been exposed to natural infestation for the previous four to five weeks.

Faecal examination

Faecal samples for worm egg counts and faecal cultures were collected monthly per rectum from the same 10 cows and in addition from February 1976 onwards from 10 heifers running with the tracer calves. The number of nematode eggs per gram of faeces (e.p.g.)[20] was determined for each of the 20 monthly faecal samples. A composite culture for larval differentiation was made from the faeces of the cows, and one from that of the heifers. Third stage larvae were identified according to Keith's key[12].

Helminth recovery

At autopsy both conjuctivae of each animal were inspected and all macroscopically visible *Thelazia rhodesii* (eyeworms) removed for counting. The animals were eviscerated and the gastro-intestinal tract separated into rumen, abomasum, and small and large intestine. All tracheae and bronchi were slit open to slightly beyond the tracheal bifurcation and inspected for the presence of lungworm. The rumens were opened and the pillars examined for the presence of adult conical fluke. After skinning, the carcasses were examined and any lesions present scrutinized for the presence of *Parafilaria bovicola.* In October 1975 the abomasal and intestinal ingesta were washed on a sieve with 150 μm apertures and the worms counted macroscopically *in toto.* From November 1975, one 1/10 aliquots of the abomasal and small intestinal ingesta were taken and washed on sieves with 38 μm apertures for microscopic counting. The remaining 9/10 of the ingesta was washed on a sieve with 150 μm apertures. The large intestin-

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al ingesta were washed on a sieve with 150 μm apertures, and one 1/10 aliquot examined microscopically for worms. If any large worms (eg. adult *H. placei, B. phlebotomum* or *O. radiatum*) were encountered in the aliquots, total macroscopic counts were done on the remaining 9/10 of the particular organs. From April 1976, the mucosae of the abomasa and 1/3 of those of the small and large intestines were removed by scraping for pepsin-HCl digestion. Total microscopic counts of the worms present in these digests were performed. All livers were cut into slices approximately 5 mm thick and each slice scrutinized for the presence of fluke.

RESULTS

Faecal worm egg counts

Faecal worm egg counts were never very high, and the highest mean recorded was 510 e.p.g. for the heifers in January (Figure 1). The heifers' egg counts consistently exceeded those of the cows, except in March when the heifers were accidentally dewormed with the rest of the breeding herd, and all were negative.

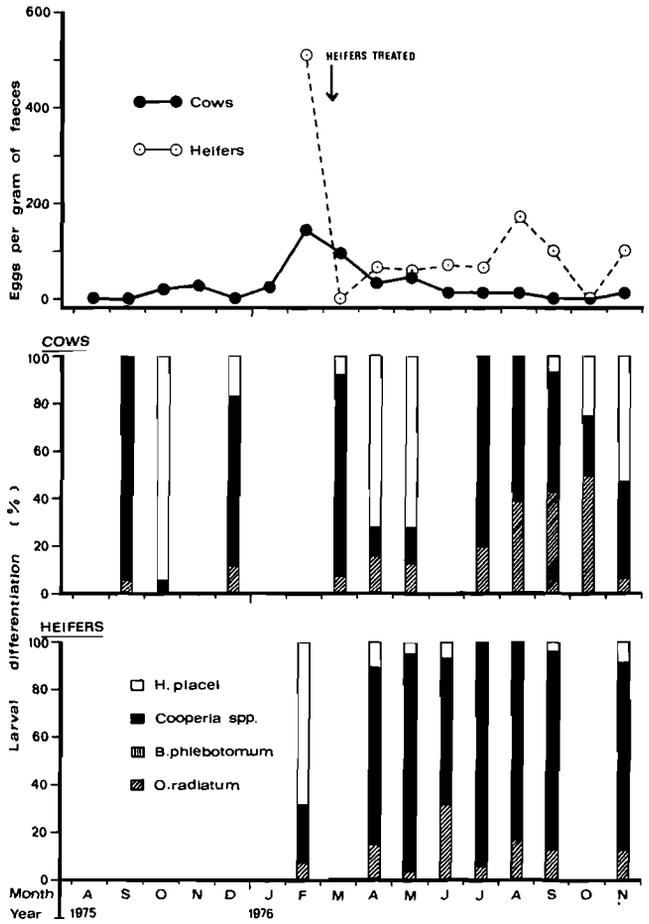

Fig. 1 Mean monthly faecal worm egg counts and larval differentiation done on the faeces of 10 cows and 10 heifers

Infective larvae

The following species were present in the faecal cultures: *Cooperia* spp., *H. placei, O. radiatum* and *B. phlebotomum*. The first three were present in both cows and heifers, but *B. phlebotomum* larvae were present only in the cultures of heifers in February and again in September, 1976. *Cooperia* spp. made the biggest contribution to the faecal output of nematode eggs, but this contribution appeared more variable in the cows than in the heifers.

Nematodes recovered at autopsy

Cooperia spp. made up the bulk of the gastro-intestinal nematodes and occurred in low numbers (90 to 1 150) from December 1975 to March 1976. Thereafter very low burdens were recorded until September 1976. Mean *Cooperia* burdens of 315 and 1 250 worms were recorded for the last two months of the trial (Table 1).

Haemonchus placei occurred in low numbers, mostly in January, February and March. It was completely absent from May to October, 1976. Only fourth stage larvae were recovered from the calves slaughtered during April and November 1976.

Oesophagostomum radiatum occurred in very low numbers throughout the trial. The highest count recorded was 38 worms from one of the calves slaughtered during January. *B. phlebotomum* was found in only three animals, namely in one of each of the calves slaughtered during January, October and November 1976.

Trichostrongylus spp. were also recovered in very low numbers and only from January to March 1976. The 40 worms recovered from one calf slaughtered in January was the highest count recorded.

Parafilaria bovicola were recovered in low numbers from tracer calves exposed during the period October 1975 to February, 1976. All specimens were adult females, as was the case with *T. rhodesii,* which occurred in the conjunctival sacs of calves slaughtered during November, 1975 and January and November 1976. Most of the *T. rhodesii* were found under the *membrana nictitans.*

Three calves were infested with *Moniezia* sp.

No lungworm, liver fluke or conical fluke were encountered.

A total rainfall of 533 mm was recorded for the 14-month trial period. The curve for mean monthly gastro-intestinal worm burdens followed that for monthly rainfall (Fig. 2).

DISCUSSION

The objective of this survey was to determine the seasonal incidence of helminth parasites in cattle in order to devise a strategic anthelmintic dosing programme. For this reason, tracer calves were employed to determine the seasonal availability of infective larvae on the pasture. A shortcoming of this method is that the cumulative worm burdens of cattle that are continuously exposed are not determined.

Horak & Louw[11] used worm-free tracer calves and this is obviously the ideal. In an endemic heartwater area the introduction of fully susceptible animals is not feasible, and the rearing of worm-free calves at the trial location was in this case impractical. The possibility of using worm-free tracers immunized against heartwater was not investigated. The development of immunity to helminth infestation in the tracer calves, which were continuously exposed before their particular terms as tracers, was a possible disadvantage of this experiment.

Table 1: TOTAL WORM BURDENS OF TRACER CALVES EXPOSED FOR PERIODS OF ONE MONTH IN THE WATERBERG DISTRICT

Calf no.	Month slaughtered	*H. placei*		*Trichostrongylus* spp.		*Cooperia* spp.				*B. phlebotomum*			*O. radiatum*				*P. bovicola*	*T. rhodesii*	*Moniezia sp.*
		L_4	Ad**	L_4	Ad	L_3**	L_4	L_5	Ad	L_4	L_5	Ad	L_3	L_4	L_5	Ad	Ad	Ad	Ad
1	October 75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3	November	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0
4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	7	7
5	December	0	0	0	0	0	0	0	90	0	0	0	0	0	0	0	0	0	0
6		0	0	0	0	0	20	120	10	0	0	0	0	0	0	4	3	0	0
7	January 76	0	886	0	10	0	0	140	510	0	4	0	0	0	14	24	0	2	2
8		0	434	10	30	0	0	70	500	0	0	0	0	0	1	4	1	0	1
9	Feb	50	359	0	0	0	80	480	590	0	0	0	0	0	0	0	3	0	0
10		20	91	0	10	0	70	330	240	0	0	0	0	0	0	17	1	0	0
11	March	10	169	0	20	0	50	240	410	0	0	0	0	0	0	1	0	0	0
12		0	25	0	0	0	80	0	120	0	0	0	0	0	0	0	0	0	0
13	April	11	0	0	0	0	0	20	0	0	0	0	0	6	2	1	0	0	0
14		8	0	0	0	0	0	50	0	0	0	0	0	3	0	0	0	0	0
15	May	0	0	0	0	9*	0	0	0	0	0	0	6	0	0	0	0	0	0
16		0	0	0	0	15*	0	20	0	0	0	0	0	9	0	0	0	0	0
17	June	0	0	0	0	18*	0	0	0	0	0	0	0	0	0	0	0	0	0
18		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	July	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
20		0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
21	August	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
22		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	September	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24		0	0	0	0	6*	0	0	0	0	0	0	0	3	0	17	0	0	0
25	October	0	0	0	0	0	16	240	40	0	0	0	0	0	0	0	0	0	0
26		0	0	0	0	10	3	300	20	3	0	0	0	0	8	0	0	0	0
27	November	73	0	0	0	250	363	560	270	0	0	0	0	0	2	0	0	7	0
28		10	0	0	0	543	173	190	150	10	0	0	0	0	4	0	0	2	0

*These larvae came from digests and could not be identified specifically
**L_3 = third stage and L_4 = fourth stage larval. 5 = fifth stage and Ad = adult worms.

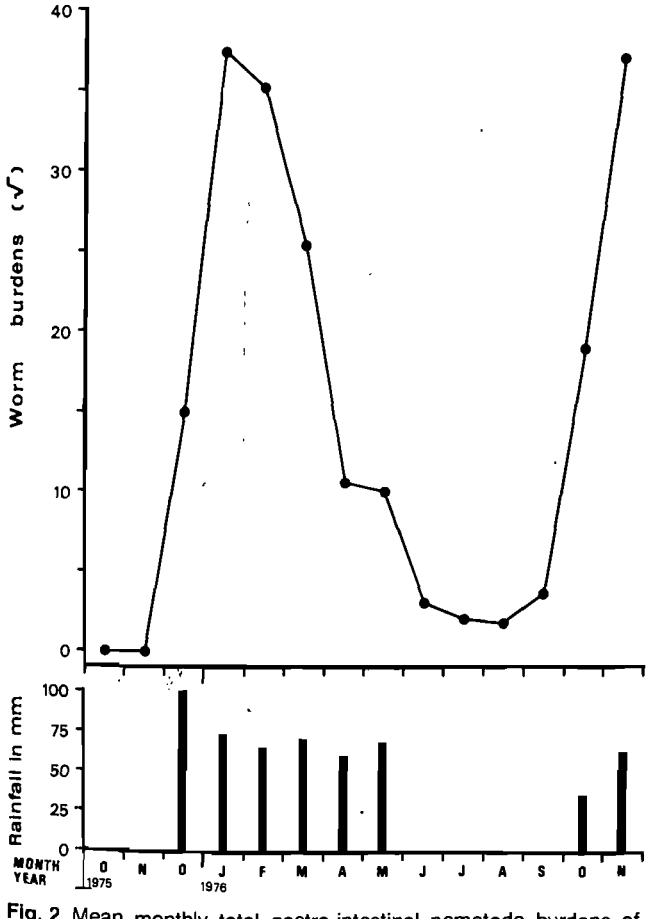

Fig. 2 Mean monthly total gastro-intestinal nematode burdens of tracer calves and rainfall in the survey area

It has been stated though, that resistance to helminths is fostered by way of premunition[30], and it has been shown that beef calves in Queensland were very susceptible to helminth infestation upon cessation of monthly drenching at weaning[5].

This seems contrary to the work of Keith[13] who showed that fortnighly drenching did not adversely influence the immunity of grazing calves if worm egg-production was used as a parameter. It seems likely that an immune state sufficient to suppress nematode egg-laying, but insufficient to prevent re-infestation is operative in these cases[30]. In this experiment the possible effect on egg-production was not investigated.

In planning the present experiment, it was thought that acquired immunity of the tracer calves would wane sufficiently during a month relatively "free" from worms, and this "worm-free" state could best be achieved under the prevailing circumstances by repeated drenching with an effective broad-spectrum anthelmintic. No attempt was made to establish the worm burdens of selected tracer calves prior to treatment, but the results tend to support the initial assumptions. Three successive drenches did in fact render the trial calves worm-free, and allowed them to become infested when larvae were available.

Faecal egg counts are valuable indicators of the species of helminths present and the rate of pasture contamination. They do not give an indication of the availability of infective material on the pasture, or of the worm burden in infested animals[4 14 17 23]. Seasonal fluctuations in faecal worm egg counts cannot be used as an

indication of similar fluctuations in worm burdens and/or rates of infestation. It is true, however, that egg-production is generally highest in summer[10 20 30], when conditions for infestation are also more favourable.

Another survey technique is that of helminth recovery in an abattoir from the viscera of cattle originating from a particular area[16 30]. It affords an estimate of total worm burdens in grazing cattle, but does not necessarily reflect seasonal availability of viable infective larvae on the pasture.

The 'seasonal incidence' of *P. bovicola* in this trial roughly corresponds to that described by Viljoen[28], who recorded a rapid increase in the numbers of clinically positive animals from June to a peak in September/October, and Nevill[18] who noticed bleeding marks between July and December. The life-cycle of this parasite is still not clear in many respects, but it seems logical that the worms are present in the subcutis some time before the adult female penetrates the skin for microfilarial release. Therefore one would have expected the recovery of *P. bovicola* in this experiment to have been made earlier than October. All *P. bovicola* in this trial were recovered from typical lesions[26], but no cutaneous haemorrhages were noticed.

No lesions were seen in the eyes in which *T. rhodesii* were found. This agrees with findings in the United Kingdom[3], but contrasts sharply with the observations in Ghana[29], where conjunctivitis and ulcerative keratitis have been attributed to this parasite. In this survey, as in Ghana[29] and the Ukraine Republic[24], *T. rhodesii* occurred mainly during the summer months. This seasonal incidence is probably directly correlated with the activity of the intermediate host (*Musca* spp.[3 29]), but it is likely that once an animal has become infested, it can remain so throughout the year[24].

Four of the gastro-intestinal nematodes recovered in this survey: *H. placei, Cooperia* spp., *O. radiatum* and *B. phlebotomum,* have been described as the most common in bovines in the North Western Cape[19]. The dry climate during the trial period was probably responsible for the low numbers of *H. placei* and the dominance of *Cooperia* spp., which are more resistant to adverse climatic conditions[15].

Although *O. radiatum* has been shown to assume considerable economic importance in a more tropical climate[16], it was only recovered in low numbers in this survey. The highest count recorded in January corresponds to an egg-count peak in February previously described in Botswana[6].

Based upon the classical epidemiological studies performed in Australia[8 22], strategic drenching programmes are generally employed for the control of internal parasites in cattle[7] and sheep[9]. If helminth infestation challenge is very low, as was the case during this survey, this procedure may prove to be unjustifiably costly. The reason for the very low worm burdens of the tracer calves is probably two-fold. In the first instance the trial location is in a region with an average annual rainfall of ca. 680 mm[2]. The total rainfall (533 mm) for the 14 months' duration of the trial was lower than this average, and could hardly have created favourable conditions for the hatching of nematode larvae and their release from dung pats. The second possible explanation is the low stocking rate (2,75 ha per large animal unit), which would preclude heavy faecal contamination of pastures and the accumulation of large numbers of infective larvae.

As gastro-intestinal worm burdens were apparently correlated with rainfall in this survey (Fig 2), a tactical regimen would appear to be more prudent than a stragetic one. A broad-spectrum anthelmintic, administered within 14 days of 40 mm of rain being measured during a two- to three-day period would most likely suffice.

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BOOK REVIEW — BOEKRESENSIE

ATLAS OF RADIOGRAPHIC ANATOMY OF THE HORSE

H. SCHEBITZ and H. WILKENS. 3rd Revised Ed.
Verlag Paul Parey, Hamburg 1978 pp. 100 Figs 128 (7 in colour) Publ. Price DM 116

In the first two editions of this Atlas the radiographic anatomy of the horse and dog were combined in one volume. The third edition however consists of two separate volumes of which the one dealing with the radiographic anatomy of the dog and cat was published in 1977 and reviewed recently.

The reader is shown how to position the horse for radiographic examination of the various anatomical regions and in every case a reproduction of the radiograph obtained is included and explained by means of an annotated line drawing. The various bones of the carpal and tarsal joints have been outlined in different colours to facilitate their identification. Positioning is explained in both German and English. The common English and German terms for anatomical structures have been discontinued and replaced throughout by the accepted nomenclature of Nomina Anatomica Veterinaria (NVA).

The following regions were examined: head – including the nasal cavity, teeth, paranasal sinusses, gutteral pouch cervical vertebral column and dorsal spinous proecesses of the thoracic vertebrae, thoracic limb from the shoulder joint and the pelvic limb from the hip joint.

New material included in the third edition are radiographs of the incisors taken with the cassette in the mouth, one radiograph of the upper cheek teeth at the age of 6 years for comparison with the previously included one taken at 12 years; a ventro-dorsal radiograph of the hip joint, and a dorsoplantar view of the hind manus.

Details of the exposure factors are supplied with each positioning. The original radiographs were mostly taken with the aid of high definition intensifying screens to ensure maximum detail. This necessitated the use of fairly high mAS values which, in quite a number of instances, will probably be beyond the capabilities of X-ray units used by most private practitioners. This however, is no serious problem as these values can be reduced by about 75% when using fast intensifying screens. The kilovoltages used were mostly within the ranges of mobile units. The exceptions here will probably be examinations of the shoulder and hip joints and to a lesser extent the elbow joint.

Although the Atlas is an aid to the identification of anatomical structures it serves an even greater purpose in helping one to decide whether the general outline or position of a specific structure is normal or not.

It is difficult to suggest any improvements to such an excellent publication. If possible the inclusion of oblique views of the carpus in flexion and extension as well as the use of different colours in the line drawings of some radiographs of the cranium should be considered.

The Atlas is regularly consulted at the Faculty and can be recommended to all practitioners who regularly, or perhaps not so regularly, have occasion to radiograph equines.

C.J.R.

THE ANTHELMINTIC EFFICACY OF ALBENDAZOLE AGAINST GASTROINTESTINAL ROUNDWORMS, TAPEWORMS, LUNGWORMS AND LIVERFLUKES IN SHEEP

P.C. VAN SCHALKWYK, T.L. GEYSER, MARGARIDA RÉCIO and F.P.G. ERASMUS

ABSTRACT: van Schalkwyk, P.C: Geyser, T.L. Récio, Margarida; Erasmus, F.P.G. **The anthelmintic efficacy of albendazole against gastrointestinal roundworms, tapeworms, lungworms, and liverflukes in sheep** *Journal of the South African Veterinary Association* (1979) **50** No 1 31 (En) SmithKline Animal Health, Terenure Research Station, P.O. Box 38, Isando 1600, Republic of South Africa.

Anthelmintic trials were carried out to evaluate the efficacy of albendazole against helmi
of 2,5 to 3,8 mg/kg administered orally, resulted in a 98,8 to 100 % reduction of adult parasites of the genera *Haemonchus, Ostertagia, Trichostrongylus, Nematodirus, Gaigeria, Oesophagostomum, Chabertia, Marshallagia* and *Cooperia.* Against the immature stages of these genera, except for *Marshallagia* and *Cooperia,* which were not tested, a dose level of 2,5 to 3,8 mg/kg was 83,9–100 % effective.

Albendazole at 2,5 mg/kg was 99,0 % effective against adult stages of *Dictyocaulus;* its activity at a dose of 3,8 mg/kg against the immature stages of *D. filaria* was 89,3 %

In sheep naturally infested with *Moniezia,* 100 % elimination was obtained at a dose level of 2,5 mg/kg.

Dose levels of 3,8 mg/kg and higher were more than 76 % effective against adult *Fasciola hepatica,* while a dose of 4,8 mg/kg was 63 % effective against adult *Fasciola gigantica.*

INTRODUCTION

Albendazole (methyl 5-propylthio-1*H*-benzimidazol carbamate) has been reported to be highly effective against artificial and naturally acquired nematode infestations of cattle[3 10]. Theodorides *et al*[9] also described its efficacy against roundworms, tapeworms, lungworms and liver fluke of sheep. In the present study the non-parametric method described by Groeneveld & Reinecke[2] and subsequently modified[6], was used to analyse the results of anthelmintic tests with albendazole against parasitic nematodes, cestodes and trematodes of sheep.

MATERIALS AND METHODS

Nematodes

A total of 150 Merino lambs was used in eight trials. All animals were treated with a commercially available anthelmintic well before commencement of the trials. Animals were kept in a closed shed with a raised expanded metal floor. This form of housing has been proven to prevent natural parasitic infestation of animals. A milled mixture of lucern and *Eragrostis* hay was provided *ad libitum* during the trials.

The larval production methods and infestation procedure described by Reinecke[6] were followed. The design of the experiments 1 to 8 is summarised in Table 1. The third (L_3) and fourth (L_4) larval stages of *Dictyocaulus filaria* were not tested separately but were combined as 1–8 day old worms in Experiment 7. Only the adult stages of *Marshallagia marshalli* and *Cooperia* spp were tested.

A 1,9 % m/v suspension of albendazole was used in all experiments except in No. 2 where the concentration of the suspension used was 5 % m/v. On the day of treatment animals were assigned by random numbers to control or treated groups. The individual live masses were measured and the appropriate quantity of drug administered by means of a stomach tube inserted into the upper oesophagus. Dosage levels of either 2,5 mg/kg or 3,8 mg/kg live mass were used.

The methods for recovery of worms *post mortem* described by Reinecke[6] were followed. The modified Baermann apparatus[7], yielding filtrate and residue samples, was used for the recovery of parasites other than the adult stages of *H. contortus, G. pachyscelis, O. columbianum* and *C. ovina.* Total worm burdens were determined miroscopically (using a stereomicroscope) for samples of filtrate and digested mucosa and macroscopically for residues. In addition a 1/10 aliquot of the residues was examined microscopically.

Table 1: DESIGN OF EXPERIMENTS

Experiment No.	Parasite tested	Stage tested	Dose level (mg/kg)
1	*H. contortus*	Adult	2,5
	N. spathiger	Adult	
	G. pachyscelis	Adult	
	C. ovina	Adult	
2	*H. contortus*	L_4	2,5
	T. colubriformis	L_4	
	C. ovina	L_3	
3	*H. contortus*	L_3	2,5
	N. spathiger	L_3	
	G. pachyscelis	L_3	
	C. ovina	L_4	
4	*O. circumcincta*	Adult	2,5
	T. colubriformis	Adult	
5	*O. circumcincta*	L_4	2,5
	N. spathiger	L_4	
	O. columbianum	L_4	
	D. filaria	Adult	
6	*O. circumcincta*	L_3	2,5
	T. colubriformis	L_3	
	G. pachyscelis	L_4	
	O. columbianum	Adult	
7	*O. columbianum*	L_3	3,8
	D. filaria	L_3 & L_4	
	M. marshalli	Adult	
8	*Cooperia* spp.	Adult	3,8

Cestodes

Sixteen Merino lambs naturally infested with *Moniezia expansa* were selected for the trial. The infestation was

0038-2809/79/01/0031

Table 2: ARTIFICIAL INFESTATION PROCEDURES

Stage tested:	L_3		L_4		Adult	
Species	Larvae*	Days**	Larvae	Days	Larvae	Days
H. contortus	1 500	–2 to –1	300	–11 to –3	300	–21 to –12
O. circumcincta	1 000	–3 to –1	500	– 9 to –4	250	–21 to –10
T. colubriformis	1 000	–3 to –1	430	–10 to –4	275	–21 to –10
N. spathiger	1 000	–3 to –1	335	–12 to –4	334	–21 to –13
G. pachyscelis	400	–6	400	–15	400	–70
O. columbianum	134	–6 to –1	55	–21 to –7	40	–42 to –22
C. ovina	100	–8 to –1	50	–25 to –9	40	–48 to –26
M. marshalli	–	–	–	–	1 000	–21 to –19
Cooperia spp.	–	–	–	–	1 000	–21 to –19
D. filaria	150	–8 to –1			48	–33 to –9

*Number of infective larvae administered per animal per day.
**Days before treatment when larvae were administered.
Treatment was applied on Day 0.

confirmed by the presence of proglottids in the faeces of all animals. The lambs were housed and fed as described in the nematode trials. On the day of treatment the lambs were weighed and allocated by random numbers to two groups of five untreated controls and eleven animals treated with albendazole at a dose rate of 2,5 mg/kg live mass. Albendazole suspension was administered intra-ruminally by means of a stomach tube.

After treatment, faeces were collected in McMaster collection bags for three days, and examined for the presence of proglottids. The volume of the eliminated strobilae was determined by displacement. All sheep were slaughtered 12 days after treatment. At autopsy the small intestine, caecum and colon were opened, the ingesta washed through a 150 μm sieve and the residue on the sieve and intestinal mucosa inspected for the presence of scoleces.

Trematodes

A total of 107 sheep was used in 4 trials.

Animals were artificially infested with metacercariae of *Fasciola hepatica* or *Fasciola gigantica*. Dose levels of 2,5 to 15 mg/kg were used to treat the animals when the flukes were 6 to 17 weeks old.

At autopsy the bile ducts were cut open and all the flukes removed. The livers were then cut into small pieces which were submerged in normal saline solution kept at 42°C. After 2 hours the liver material was removed from the saline and washed by hand to recover all flukes.

Specimens were examined macroscopically.

RESULTS

Nematodes

The numbers of nematodes recovered are summarised in Tables 3 to 7.

Albendazole at a dose rate of 2,5 to 3,8 mg/kg was 98,8 to 100 % effective in removing adult stages. Similar efficacy was obtained against L_3 and L_4 with exception of L_3 of *O. columbianum* (84 %) and of L_3 and L_4 *D. filaria* (89 %).

Cestodes

Following treatment 10 of 11 treated animals passed strobilae with volumes ranging from 15 to 77 mℓ. At

Table 3: WORMS RECOVERED AND EFFICACY OF ALBENDAZOLE

Species:	*Haemonchus contortus*			*Ostertagia circumcincta*		
Stage:	L_3	L_4	Adult	L_3	L_4	Adult
9 Controls	635	358	737	424	1295	1344
	868	395	979	707	1537	1578
	944	419	981	710	1557	1616
	1016	557	1033	784	1702	1683
	1249	564	1042	824	1775	1722
	1274	585	1168	871	1934	1951
	1348	638	1493	1022	2044	1996
	1368	708	1515	1231	2113	2537
	1668	709	1543	1233	2169	2623
Group mean	1152,2	548,1	1165,7	867,3	1791,8	1894,4
11 Treated Albendazole 2,5 mg/kg	0	0	0	1	0	0
	0	0	0	1	0	3
	0	0	0	2	0	4
	0	0	0	2	0	5
	0	0	0	2	0	7
	0	0	0	3	0	8
	0	0	0	3	0	14
	0	0	0	4	0	15
	0	0	0	5	1	38
	8	1	0	7	2	40
	12	1	0	8	2	48
Group mean	1,8	0,2	0	3,5	0,5	16,5
% Reduction	99,8	99,9	100	99, 6	9 9,9	99,1
NPM Class	A	A	A	A	A	A

Figures that are underlined indicate the median.

autopsy all the control animals were infested with *Moniezia expansa* while all treated animals were negative (Table 8).

Trematodes

The efficacy of albendazole against *F. hepatica* and *F. gigantica* is summarised in Tables 9 and 10.

DISCUSSION

Albendazole is the first anthelmintic benzimidazole reported to have a useful activity against nematodes, cestodes and trematodes of ruminants[5 8 11 13].

Since the discovery of the first anthelmintic of this series, thiabendazole[1], other benzimidazole compounds have been introduced with activity against nematodes and, in some cases, also against cestodes. The history of the introduction of these anthelmintics follows a pattern of increasing potency and spectrum. Thiabendazole is registered in South Africa as an anthelmintic effective against gastrointestinal roundworms and lungworms at dose levels of 44 and 88 mg/kg; parbendazole against gastrointestinal roundworms at 30 mg/kg and tapeworms at 60 mg/kg; cambendazole against gastrointestinal roundworms and tapeworms at 20 mg/kg; and mebendazole against gastrointestinal roundworms, lungworms, and tapeworms at 15 mg/kg. Fenbendazole is registered for use against gastrointestinal roundworms and lungworms at 5,0 mg/kg and is reportedly highly active against tapeworms at 10 mg/kg[11].

Albendazole is highly active against gastrointestinal roundworms, lungworms, tapeworms and liver flukes at dose rates of 2,5 and 5,0 mg/kg. This spectrum of ef-

Table 5: WORMS RECOVERED AND EFFICACY OF ALBENDAZOLE

Species:	*Gaigeria pachyscelis*			*Chabertia ovina*		
Stage:	L_3	L_4	Adult	L_3	L_4	Adult
9 Controls	52	60	107	199	75	568
	66	114	176	205	220	644
	125	128	207	221	479	656
	126	194	214	235	534	689
	145	215	217	311	683	693
	146	217	234	354	689	702
	164	227	242	404	702	707
	201	245	255	636	717	811
	227	248	259	683	779	814
Group mean	139,1	183,1	212,3	360,9	542,0	698,2
11 Treated Albendazole 2,5 mg/kg	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	1	0
	0	0	0	0	1	0
	0	0	0	0	1	0
	0	0	0	0	1	0
	1	0	0	1	1	0
	1	0	0	1	1	0
	3	0	0	1	3	0
Group mean	0,5	0	0	0,3	0, 8	0
% Reduction	99,6	100	100	99,9	99, 9	100
NPM Class	A	A	A	A	A	A

Table 4: WORMS RECOVERED AND EFFICACY OF ALBENDAZOLE

Species:	*Trichostrongylus colubriformis*			*Namatodirus spathiger*		
Stage:	L_3	L_4	Adult	L_3	L_4	Adult
9 Controls	1447	1592	304	89	100	6
	1637	1624	326	312	113	193
	1704	1662	329	358	440	201
	1754	1667	333	434	782	550
	1806	1694	355	977	803	633
	2016	1696	364	1044	1382	697
	2180	1950	374	1076	1403	1017
	2191	2141	380	1079	1612	1074
	2273	2145	389	1268	1879	1506
Group mean	1889,8	1796,8	350,4	737,4	946,0	653,0
11 Treated Albendazole 2,5 mg/kg	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	1	0	0
	0	0	0	1	0	0
	0	0	0	1	0	0
	0	0	0	3	0	0
	0	0	0	4	0	0
	0	0	0	10	0	0
	0	0	0	13	0	0
Group mean	0	0	0	3,0	0	0
% Reduction	100	100	100	99,6	100	100
NPM Class	A	A	A	A	A	A

Table 6: WORMS RECOVERED AND EFFICACY OF ALBENDAZOLE

Species:	*Oesophagostomum columbianum*		*Dictyocaulus filaria*
Stage:	L_4	Adult	Adult
9 Controls	80	389	41
	194	473	69
	246	515	106
	258	532	142
	378	654	165
	386	664	174
	450	702	178
	459	705	192
	527	763	224
Group mean	330,9	599,7	143,4
11 Treated Albendazole 2,5 mg/kg	1	0	0
	12	0	0
	15	1	1
	25	1	1
	27	2	1
	27	2	1
	28	7	1
	29	8	1
	47	11	2
	52	18	3
	65	29	6
Group mean	29,8	7,2	1,5
% Reduction	91,0	98,8	99,0
NPM Class	A	A	A

Table 7: WORMS RECOVERED AND EFFICACY OF ALBENDAZOLE

Species:	*O. columbianum*	*D. filaria*	*M. marshalli*	*Cooperia* spp.
Stage	L_3	Immatures (L_3 and L_4	Adult	Adult
Controls	263	27	127	70
	294	56	128	98
	425	91	333	832
	473	111	396	1303
	566	186	636	1348
	654	362	–	–
	750	385	–	–
	751	443	–	–
	764	444	–	–
Group mean	548, 9	2 3 3, 9	3 2 4,0	730,2
Group mean	548, 9	2 3 3, 9	3 2 4,0	730,2
Treated Albendazole 3,8 mg/kg	51	6	0	0
	64	9	0	0
	64	11	0	0
	75	12	0	0
	75	14	0	3
	80	17	–	–
	82	20	–	–
	103	29	–	–
	103	36	–	–
	116	38	–	–
	156	84	–	–
Group mean	88,1	251,	0	0,6
% Reduction	83,9	89,3	100	99,9
NPM Class	A	A	–	–

Table 8: EFFICACY OF ALBENDAZOLE AGAINST *MONIEZIA EXPANSA*

	Volume of strobilae excreted (ml)			Autopsy
Days after treatment:	1	2	3	12
5 Untreated controls	+	+	+	Positive
	+	+	+	Positive
	+	+	+	Positive
	+	+	+	Positive
	+	+	+	Positive
11 Treated with Albendazole at 2,5 mg/kg	13,0	11,0	+	Negative
	77,0	+	–	Negative
	39,0	+	–	Negative
	30,0	+	+	Negative
	50,0	–	–	Negative
	30,0	+	–	Negative
	15,0	–	–	Negative
	50,0	+	+	Negative
	+	–	–	Negative
	15,0	+	–	Negative
	37,0	+	–	Negative

+Only segments present in the faeces.

ficacy has a practical benefit in herd dosing programmes. According to van Wyk[12], who listed the most important helminth parasites in South Africa, the most common parasites or those that have a serious effect upon sheep are *Haemonchus, Trichostrongylus, Gaigeria* and *Oesophagostomum,* but gastrointestinal nematodes as well as lungworms, liver flukes and tapeworms are also common in certain areas of the country.

Table 9: EFFICACY OF ALBENDAZOLE AGAINST *F. HEPATICA*

Age of flukes	Animals per group	Dose (mg/kg)	Mean flukes recovered	% Reduction
17 weeks	9	Control	119,4	–
	8	5,0	7,0	94,1
	11	7,5	3,2	97,3
	11	10,0	1,8	98,5
	7	15,0	0,1	99,9
12 weeks	5	Control	52,4	–
	5	2,5	22,8	56,5
	5	3,8	12,4	76,3
6 weeks	5	Control	62,8	–
	5	2,5	53,6	14,6
	5	3,8	33,2	47,1

Table 10: EFFICACY OF ALBENDAZOLE AGAINST *F. GIGANTICA*

Age of flukes	Animals per group	Dose (mg/kg)	Mean flukes recovered	% Reduction
14 weeks	9	Control	80,7	–
	11	4,8	29,7	63,2
8 weeks	9	Control	80,7	–
	11	4,8	80,5	0

Albendazole, due to its exceptional spectrum of efficacy, should be particularly useful in dosing programmes aimed at controlling a wide range of helminth parasites.

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INFORMATION — INLIGTING

VULTURES AS CARRIERS OF ANTHRAX

A research programme headed by Dr. V.de Vos, Veterinary Ecologist of the National Parks Board, has been undertaken to discover the means whereby anthrax in wild animal populations is spread in the Kruger National Park. The research team proved beyond doubt that vultures play a complex but important role in the epizootiology of the disease which constitutes a serious threat to the Park's population of game.

Anthrax is an acute, infectious bacterial disease with worldwide distribution, affecting warm-blooded animals and resulting in fatal septicaemia. It is caused by rod-shaped bacteria *Bacillus anthracis,* which are found in the bood and organs of an infected animal. When exposed to air under favourable conditions the bacilli form spores which retain their vitality in soil, water, hides and vegetation for many years. Spores of *B. anthracis* preserved on filter paper retained their virulence after storage for nearly 70 years.

The decimating effect of anthrax was dramatically demonstrated during the 1959/60, 1962 and 1970 anthrax epizootics. During these outbreaks in the Park about 1 500 animals, representing 22 species, succumbed to the disease. This figure includes 83 roan antelope, a notoriously rare species totalling not more than 350 in the whole of the Republic of South Africa.

The Pafuri area of the Kruger National Park is regarded as being an enzootic anthrax region in that the disease spread sporadically from there to adjoining regions to set up foci of infection which may flare up as epizootics. The rest of the Park therefore, is exposed to the constant and dreaded threat of anthrax. The research programme headed by Dr. de Vos is aimed at preventing the spread of the disease to other areas of the Park.

As vultures have acquired the unfavourable reputation of being one of the chief distributors of the disease, part of the programme was directed to their role in the ecology of anthrax in the Park. This included studies on behavioural activities, backed by experimental bacteriological research.

Vultures visit watering places immediately after gorging themselves on dead animals, in order to bathe and drink. Here they wash off the blood adhering to their feathers and also sometimes regurgitate ingested material infected with anthrax bacilli into the water or along the edges, thereby forming an ideal means of transmission to other animals. In this manner natural waterholes and drinking troughs become infected by vultures during an outbreak of the disease.

The vulture's role as a potential carrier was further proved under experimental laboratory conditions when vultures were fed with anthrax spores and vegetative forms. Anthrax bacilli were isolated from faecal matter of spore-fed vultures for a period of up to two weeks. No anthrax could, however, be isolated from faeces of vultures fed with the pure vegetative forms of anthrax.

The next step was to assess the distance over which dissemination of the disease may take place. The birds were cannonnetted and ring-marked in collaboration with interested parties from Rhodesia and the Vulture Study Group of the South African Ornithological Society. Preliminary results showed a very wide flying range, especially for the young white-backed vulture, *Gyps africanus.* A bird ringed near Skukuza in the Kruger National Park was sighted at Hluhluwe in Natal a few months later and another ringed at Kariba, Rhodesia, was sighted in Swaziland. By virtue of their scavenging habits and flight distances, vultures are therefore able to spread anthrax over vast areas.

On the other hand, the vulture plays a vital role in the early detection and disposal of carcasses which may constitute a positive aspect of limiting anthrax infection. In cases where impala carcasses were put out, most of the soft material was devoured within one hour after the carcass was opened up, and totally devoured within half a day. In nature sporulation occurs only after a carcass has been opened and exposed to the air for several hours after favourable conditions. The quick and efficient manner of disposal by vultures, therefore, greatly reduces the amount of infective material, if it is kept in mind that the digestive system of the vulture also effectively disposes of the vegetative form.

The study points to the vulture as having a dual role in the ecology of anthrax. While it may spread the disease over large areas, it does at the same time effectively reduce the pathogen crop in nature.

Die mees belangrike 21 dae in 'n koei se jaar.

Die Probleem.

'n Hoë persentasie koeie het 'n residuele infeksie aan die einde van laktasie.

Daarby is alle koeie vatbaar vir 'n nuwe infeksie gedurende die vroeë stadia van die droë periode. Met 'n paar uitsonderings sal dit gedurende die eerste 10 tot 21 dae voorkom.

Die Antwoord.

Orbenin Droë Koei is ontwerp om beide hierdie probleme te oorbrug. Dit is geformuleer as gevolg van aanhoudende navorsing, beide in die laboratorium en in die veld.*

Orbenin Droë Koei is bakteriedodend teen streptokokke, penisillien sensitiewe en penisillien weerstandbiedende staphylokokke, die mees oorsaaklike organismes wat in residuele en nuwe infeksies gevind word.

Dit was duidelik uitgewys dat infeksie tydens kalwing gewoonlik van NUWE oorsprong en meesal 'n omgewings, of Gram -we infeksie is. Behandeling tydens daardie tyd met 'n breëspektrum Lakterende Koei produk soos Ampiclox L.K. is nodig om genoegsame antibiotiese konsentrasies te bereik om mastitis tydens kalwing te voorkom.

Orbenin Lakterende Koei en Ampiclox L.K. is bedoel om mastitis gedurende laktasie te beheer. Ampiclox word veral aanbeveel wanneer coliforme vermoed word.

Orbenin (kloksasillien) en Ampiclox (ampisillien/kloksasillien) is Beecham handelsmerke.

*Die jongste kliniese proef het 507 kuddes oor 'n tydperk van 3 jaar ingesluit – Brander G.C., Watkins J.H., en Gard R.P., Vet Rec. (1975) 97. 300-304.

Beecham Dieregesondheid **Vordering in die Praktyk**

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PLASMA PROGESTERONE LEVELS IN PROGESTERONE TREATED COWS

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ABSTRACT: Grosskopf, J.F.W.; van Niekerk, C.H.; Morgenthal, J.C. **Plasma progesterone levels in progesterone treated cows.** *Journal of the South African Veterinary Association* (1979) **50** No. 1, 37 (En) Faculty of Veterinary Science, University of Pretoria, 0110 Onderstepoort, Republic of South Africa.

A technique for the radioimmunoassay of progesterone in plasma is described. In one trial the oestrous cycles of four cycling cows and in another trial of one non-cycling cow and two cycling heifers were synchronized by the administration of progesterone. Each female received either 50 mg or 0,1 mg/kg of progesterone intramuscularly on alternate days in two courses of four and six injections respectively. Blood samples of the animals were collected either daily or two-daily before, over the entire period of treatment and for eight days after the last progesterone injection. The results of the progesterone assays are represented graphically for each individual cow or heifer. The plasma progesterone levels during treatment were maintained reasonably well at levels corresponding to those normally encountered during the luteal phase of the cycle. The progesterone levels, however, did not drop as rapidly as desired after the last injection but might have been influenced by a residual corpus luteum from a previous ovulation.

INTRODUCTION

It is important to know how dosage level, intervals between administrations and routes of administration will influence the blood levels of progesterone when the oestrous cycles of cows are manipulated by progesterone treatment. At present very little is known about the progesterone levels in plasma during synchronization of oestrus. Lamond, Dickey, Henricks, Hill & Leland[8] measured progesterone levels in venous plasma of melengestrol treated heifers as from the 15th day of the cycle. They found that in 9 out of 10 heifers the progesterone levels dropped from the pretreatment levels of between 3 and 10 ng per ml to below 2 ng per mℓ of plasma within the first three days of treatment. In another trial[7] eight lactating beef cows were intramuscularly injected with 100 mg of progesterone every 48h for eight or ten times. With the last progesterone injection 2000 I.U. of PMSG was also administered to induce multi-ovulations. Unfortunately the hormone levels of only two cows and only during the last 96h before oestrus are presented.

Hansel *et al.*[6] assayed the progesterone concentrations in the plasma of Holstein heifers which had been treated with MAP (6-methyl-17-acetoxyprogesterone) in their drinking water over a 24 day period followed by a single oestradiol benzoate injection. The MAP treatment had an immediate depressing effect on the progesterone levels but after the fourth day of treatment the progesterone again rose to a level of between 2 and 3 ng per mℓ plasma in all the treated animals bar two which ovulated during the period of treatment.

Progesterone assays have, however, been performed more frequently on the blood of cows during the normal sexual cycle[1 3 12]. All these authors found that the progesterone level in the peripheral plasma dropped to below 1 ng per mℓ during estrus and in most cases even the day before oestrus. During the luteal phase the progesterone level normally rises to between 4 and 10 ng per mℓ and in exceptional cases to above 12 ng per mℓ plasma. The highest levels are usually attained between the eighth and tenth day after oestrus. Before the subsequent oestrus the level drops from approximately 6 to 8 ng per mℓ to below 1 ng per mℓ plasma within a period of three to four days.

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It appears that progesterone assays in different laboratories are not necessarily comparable. The relatively low highest values of less than 3 ng per mℓ reported by Hansel *et al.*[6] as compared to the normal levels of 8 to 10 ng per mℓ found by Hendricks *et al.*[7] and Glencross *et al.*[3] during the luteal phase serve as examples. Great variations between animals and also in the plasma of individual animals on consecutive days can also be seen in the results of the authors concerned.

At first spectrophotometric and gas-liquid chromatographic methods were employed to assay progesterone in body fluids[9 16]. Subsequently the competitive protein binding method was developed by Murphy[10] and lately the radioimmunoassay method[2 17] is favoured. Because of its exceptional sensitivity and specificity the latter method was employed for these trials.

MATERIALS AND METHODS

The progesterone levels were assayed in the plasma of two groups of cows/heifers subjected to synchronization programmes. The method of synchronization was the one described by Grosskopf[4 5] and consisted of two courses of four and six 48-hourly progesterone injections respectively with a rest period of eight days between the two courses.

Experimental animals

In the first trial four cycling lactating cows were bled at 24h or 48h intervals commencing on a day that they were found in oestrus. Blood samples were taken on Days 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17 and 19 of their cycles and then daily till the subsequent oestrous period. After the fourth cow had completed her cycle four further blood samples were again collected from each cow at 48h intervals before the commencement of the progesterone treatment. The progesterone was injected intramuscularly at 48-hours intervals at a dosage level of 50 mg in 3 mℓ of propylene glycol per injection. After the fourth injection the cows were left untreated for a period of eight days which was then followed by a second course of six 48-hourly progesterone injections at the same dosage rate. During the first course of progesterone the cows were at first bled daily and thereafter every second day till the third day after the fourth injection. As the blood sampling technique disturbed

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the cows markedly it was discontinued for a few days and resumed the day before the second course of progesterone treatment. They were again bled at 24-hourly or 48-hourly intervals throughout the treatment period and then daily during the eight days after discontinuation of the treatment during which time the onset of oestrus was anticipated.

The cows were inseminated during this latter synchronized oestrous period approximately half a day after first being observed. Insemination was repeated if a cow still showed signs of oestrus 24h after the first insemination.

Rectal ovarian palpations were carried out on the second, third and fourth days after the last progesterone injections. The mass of the cows at the end of the progesterone treatment were as follows: Cow 5, 524 kg; Cow 8, 588 kg; Cow 11, 575 kg and Cow 79, 615 kg. All four cows calved more than four months before the onset of treatment and had been in oestrus several times during that period.

In the second trial one lactating non-cycling cow (Cow 109) and two heifers (Heifer 5 and 84) were treated with progesterone (0,1 mg/kg body mass) similarly to those in the previous trial. The cow suckled her third calf which was 65 days old at the onset of treatment. Heifer 84 was 17 months old and was seen on heat for the first time approximately two months before the trial. Heifer 5 was 15 months old and was in oestrus 14 days before the onset of treatment. The mass of the animals at the onset and after the trial were respectively as follows:

Cow 109: 441 kg, 437 kg
Cow 5 : 417 kg, 411 kg
Cow 84 : 316 kg, 335 kg

Blood samples of these females were collected daily at approximately 08h00 as from the day prior to treatment until the seventh day after the last progesterone injection and then again at intervals of 2, 4, 3 and 3 days thereafter. On the third day after the fourth progesterone injection and on the third and fourth days after the last progesterone injection (the days on which oestrus was anticipated) samples were also taken during the afternoons.

Unfortunately Cow 109 died unexpectedly of bloat on the sixth day after the last progesterone injection.

Treatment of blood samples

Approximately 18 mℓ of blood was withdrawn from the Vena jugularis directly into heparinized centrifuge tubes. After centrifugation 8 mℓ of plasma was pipetted off, sealed in clean vials and stored at –15°C for assay at a later stage.

Assay of progesterone

The progesterone levels in the plasma were estimated by radioimmunoassay without prior purification. The procedure was based on the method of Morgenthal (personal communication, 1974) with some modifications.

Cleaning of laboratory glassware

All glassware used was subjected to a strict washing procedure. This was found to be absolutely essential to avoid contamination and erratic or inconsistent results. The detergent used was a 5% solution of Extran (Merck) and subsequent to repeated rinsing procedures the test tubes were also autoclaved at 135°C for 15 min. Before use all glassware was rinsed with ether as a final security measure. Automatic pipettes with disposable tips were used throughout and scintillation vials were used once only.

Reagents

1 A phosphate buffer solution (Titrisol, Merck) (pH 7,00) containing in addition 0,9% NaCl, 0,1% NaN_3 and 0,1% gelatin was used for dissolving the progesterone standards, isotope, antiserum and plasma extracts and for suspending the dextran coated charcoal.
2 The progesterone standards were prepared by serial dilution of progesterone (Merck) first in methanol and ultimately in phosphate buffer to give solutions containing 1 ng, 2 ng, 3 ng, 4 ng and 5 ng per 200 μℓ of buffer. These solutions were stored at 5°C and were replaced every two weeks.
3 The antiserum against progesterone-11-hemisuccinide bovin serum albumen was prepared by Dr J C Morgenthal, Department of Human and Animal Physiology, University of Stellenbosch, according to the method of Furr[2] and kindly donated by him. It was stored in aliquots of 0,2 mℓ at –15°C and diluted 1:10 000 for use. The diluted antiserum was found to maintain its titre for several months when stored at 5°C.
4 The progesterone isotope (Progesterone –1,2–^{3}H(N), New England Nuclear) was dissolved in phsophate buffer to an activity of approximately 15 000 to 20 000 counts per minute (c.p.m.) in 0,2 mℓ and stored at 5°C for further use.
5 Separation of the bound and free hormones was achieved by adsorption of the free hormones to dextran-coated charcoal. The charcoal suspension was prepared by suspending 1 g of activated charcoal (Merck) in approximately 100mℓ of phosphate buffer and adding to it 0,01 g Dextran T70 (Pharmacia) in approximately 50 mℓ of buffer solution. The final volume was then adjusted to 200 mℓ . The suspension was stored at 5°C and was taken out immediately before use when it was brought into homogenous suspension by a magnetic stirrer. The remaining suspension was discarded every two weeks and replaced by a fresh one.
6 The scintillation medium used was Aquagel (Chemlab). It was stored in the dark.

Extraction of steroids from plasma

The plasma samples were thawed, an aliquot taken for assay and immediately returned to the deep freeze. Of each sample 0,5 mℓ was taken and added to 10 mℓ of freshly distilled ether (Natal Cane Byproducts). A standard procedure of shaking for 1 minute on a vortex test tube shaker with intermittent shaking by hand was followed throughout. It was then left standing for a few minutes to allow separation of the ether and water phases. Thereafter the tubes were put into alcohol and dry ice for freezing and the ether fraction decanted into 15 mℓ conical centrifuge tubes. The ether was evaporated in a water bath at 38°C under a controlled stream of dry air. As control a sample of ether alone was sub-

jected to the same procedure with each batch of samples. The dried ether extracts were redissolved in 0,2 mℓ of buffer solution by shaking on the vortex test tube shaker.

The binding reaction

Standard progesterone solutions were put into thirteen 15 mℓ conical centrifuge tubes so that three tubes each contained 0,2 mℓ of phosphate buffer as the zero standards and the other ten tubes each 0,2 mℓ of the 1 ng, 2 ng, 3 ng, 4 ng and 5 ng progesterone standards in duplicate. Henceforth the tubes containing the progesterone standards and those containing the plasma extracts were similarly treated. Centrifuge space was the limiting factor which determined the number of plasma samples to be included in each batch. The centrifuge used could house 36 tubes so that 22 samples, one ether blank and 13 progesterone standards could be treated in one batch. The standard tubes were so arranged that one series was treated before the samples and their duplicates after the samples. Care was taken to subject all tubes for the same length of time to every step in the procedure.

To each tube was added 0,2 mℓ of progesterone antiserum (1:10 000) and the contents well mixed on a test tube shaker. Thereafter 0,2 mℓ of progesterone isotope was added to each tube and again well mixed. The tubes were then incubated in a water bath at 38°C for exactly 10 minutes and transferred to an ice bath at 4°C for equilibration. After 10 minutes in the cold 0,5 mℓ of the dextran-coated charcoal suspension was added and the contents mixed again by shaking. After a further 5 minutes in the ice bath the tubes were transferred to a centrifuge in polystirene containers for thermal insulation and centrifuged for 5 min at 2 000 r.p.m. (1 050 G).

For counting purposes 0,5 mℓ of the clear supernate was transferred to plastic scintillation vials with 6 mℓ of scintillation fluid.

Scintillation counting and calculations

The bound tritiated progesterone was determined by scintillation counting in a Packard Tricarb liquid Scintillation Spectrometer. Each sample was counted for 20 minutes to allow for a standard error of less than 1 %

For each batch of assays, a standard displacement curve was drawn by plotting the percentage of bound tritiated hormone against the progesterone concentrations of the standard solutions. The average count of the three zero standards were taken as the 100 % binding of tritiated hormone. Fig 1 represents the mean of 47 different displacement curves with standard deviations between curves.

The progesterone concentrations of the plasma extracts were then estimated by expressing the counts per minute obtained for each sample as a percentage of the mean zero standard's count and then by reading the progesterone level from the displacement curve for the particular batch. The assay of every sample was repeated on another day and provided the answers differed by less than 15 %, they were accepted. In those cases where the progesterone levels were higher than 4 ng per 0,5 mℓ smaller samples of plasma (0,2 mℓ or in exceptional cases even 0,1 mℓ) had to be used for extraction.

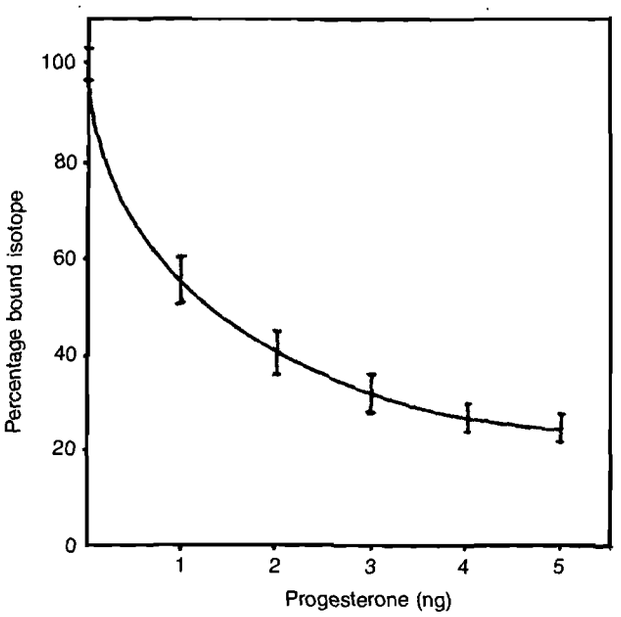

Fig. 1. The mean standard displacement curve. (Vertical bars represent the standard deviations between 47 curves).

Tests for reliability of method

The coefficient of variation between duplicate progesterone standards was tested according to a formula suggested by Murphy[10]:

$$S = \sqrt{\frac{\Sigma (d)^2}{2n}}$$

where d = difference between duplicate pairs and where n = number of duplicate pairs

This coefficient of variation were as follows for the different levels of progesterone:
0 ng: S = 2,62 per cent
1 ng: S = 2,72 per cent
2 ng: S = 2,54 per cent (n = 47 at all levels)
3 ng: S = 1,68 per cent
4 ng: S = 1,34 per cent
5 ng: S = 1,33 per cent

The effect of the different volumes of plasma used was tested and it was found that the smaller volumes gave higher readings when expressed on a per mℓ basis as shown in Table 1.

Table 1: THE PROGESTERONE CONTENT OF A PLASMA SAMPLE AS ASSAYED BY USING DIFFERENT VOLUMES OF PLASMA

Volume of sample	Progesterone content (ng)	Progesterone ng/mℓ
0,1 mℓ	1,13	11,3
0,2 mℓ	1,90	9,5
0,3 mℓ	2,56	8,5
0,4 mℓ	3,16	7,9
0,5 mℓ	4,00	8,0

This discrepency was apprently due to a small false blank value which was aggravated by extrapolation. At the higher levels of progesterone (more than 8 ng/mℓ)

where smaller volumes of plasma had to be used, these errors were of minor significance.

The lowest concentration of progesterone that could be determined with confidence was also estimated. Seven replicates of 0,0 ng, 0,1 ng, 0,2 ng, 0,3 ng and 0,4 ng and three replicates of 0,5 ng of progesterone, each in 0,2 mℓ of phosphate buffer, were prepared and subjected to the assay procedure without ether extraction. The results are shown in Table 2.

Table 2: THE ESTIMATED PROGESTERONE LEVELS IN STANDARD SOLUTIONS WITH LOW PROGESTERONE CONTENT WITHOUT ETHER EXTRACTION

Actual progesterone (ng)	Estimated progesterone (ng)	Stand. deviation
0,1	0,07	0,04
0,2	0,20	0,03
0,3	0,27	0,04
0,4	0,40	0,04
0,5	0,54	0,04

The reliability of these results were tested by an analysis of variance[13] and it was found that progesterone levels of 0,2 ng or more could significantly ($p < 0,01$) be distinguished from the zero level. It proved that they assay was extremely sensitive and accurate.

A similar test was carried out on standard progesterone solutions subjected to the ether extraction procedure described above. The results are presented in Table 3.

Table 3: THE ESTIMATED PROGESTERONE LEVELS IN STANDARD SOLUTIONS WITH LOW PROGESTERONE CONTENT AFTER ETHER EXTRACTION

Actual progesterone (ng)	Estimated progesterone (ng)	Stand. deviation
0,0	0,34	0,13
0,1	0,29	0,15
0,2	0,45	0,17
0,3	0,45	0,22
0,4	0,53	0,26
0,5	0,65	0,17
0,6	0,74	0,08

These results were similarly tested for reliability by an analysis of variance and it was found that the 0,4 ng and 0,5 ng levels of progesterone could significantly ($p > 0,05$) be distinguished from the ether extracted zero standards. It appears that the false blank value, referred to above, was in some way caused by the extraction process.

The recovery rate of progesterone was tested by adding 1,25 ng and 2,50 ng of progesterone in triplicate to aliquots of bovine plasma stripped of endogenous steroids by incubation with dextran-coated charcoal for 30 minutes at 38°C and subsequent centrifugation. The mean recovery rate was 94,5 ± 1,8%.

All these tests proved that this method of radioimmunoassay, whereby the binding reaction was carried out at 38°C for only 10 minutes was as reliable as those where a longer incubation period at lower temperatures was used. The method was therefore sufficiently sensitive for the purpose.

The values presented were not corrected for procedural losses or possible false positive readings.

RESULTS AND DISCUSSION

There was no doubt that the regular bleeding procedures did upset the animals. This might have influenced their hormonal balances to some extent as was, for example, reflected by their longer cycle lengths.

Occurence of oestrus

As mentioned, the first blood samples were collected from the cows in Trial 1 on a day that they were in oestrus. Their subsequent oestrous periods followed 21, 22, 23 and 24 days later respectively. During the rest period between the two courses of progesterone treatment three of the four animals (Cow 5, 8 and 11) showed signs of heat. During the expected synchronized oestrous period three cows came in oestrous viz. Cow 5 on the sixth day, Cow 8 on the 8th, 9th and 10th days and Cow 79 on the 3rd and 4th days after the last progesterone injection. Cow 5 and Cow 79 were fertilized by insemination during this period.

In Trial 2 Heifer 5 came on heat during the rest period as well as during the anticipated synchronized oestrous period. Heifer 84 was seen on heat for the first time on the 26th day after the last progesterone injection i.e. one cycle length after the synchronized oestrous period. The heifers were not inseminated.

Ovarian palpations

In Trial 1 rectal ovarian palpations revealed the following;

Cow 5: Developing follicle on left ovary on the 2nd, 3rd and 4th days after the last progesterone injection. Right ovary small.

Cow 8: Both ovaries large, bilateral salpyngitis, developing follicle on right ovary on 4th day after the last progesterone injection.

Cow 11: Corpus luteum on right ovary on 2nd, 3rd and 4th days after last progesterone injection.

Cow 79: Developing follicle on 2nd and 3rd days and large follicle on 4th day.

Progesterone assays

The results of the progesterone assays on the jugular plasma of the individual cows and heifers are presented in Fig 2 and 3. Each point on the graphs represents the mean of two values. The days on which progesterone was administered and those on which oestrus was observed are also indicated.

Although not identical the four graphs in Fig 2 have a similar pattern. During the luteal phase of the normal cycles prior to treatment the progesterone rose to levels varying between approximately 6 and 10 ng per mℓ of plasma. In one instance a peak level of 12,5 ng per mℓ was reached (Cow 79). The lowest levels of progesterone were found on the first and second days after oestrus. The findings correspond to those of other authors[1] [15]. However, the progesterone levels never dropped to below 1 ng per mℓ as is commonly quoted in the literature. The blank value described above could have been responsible for this.

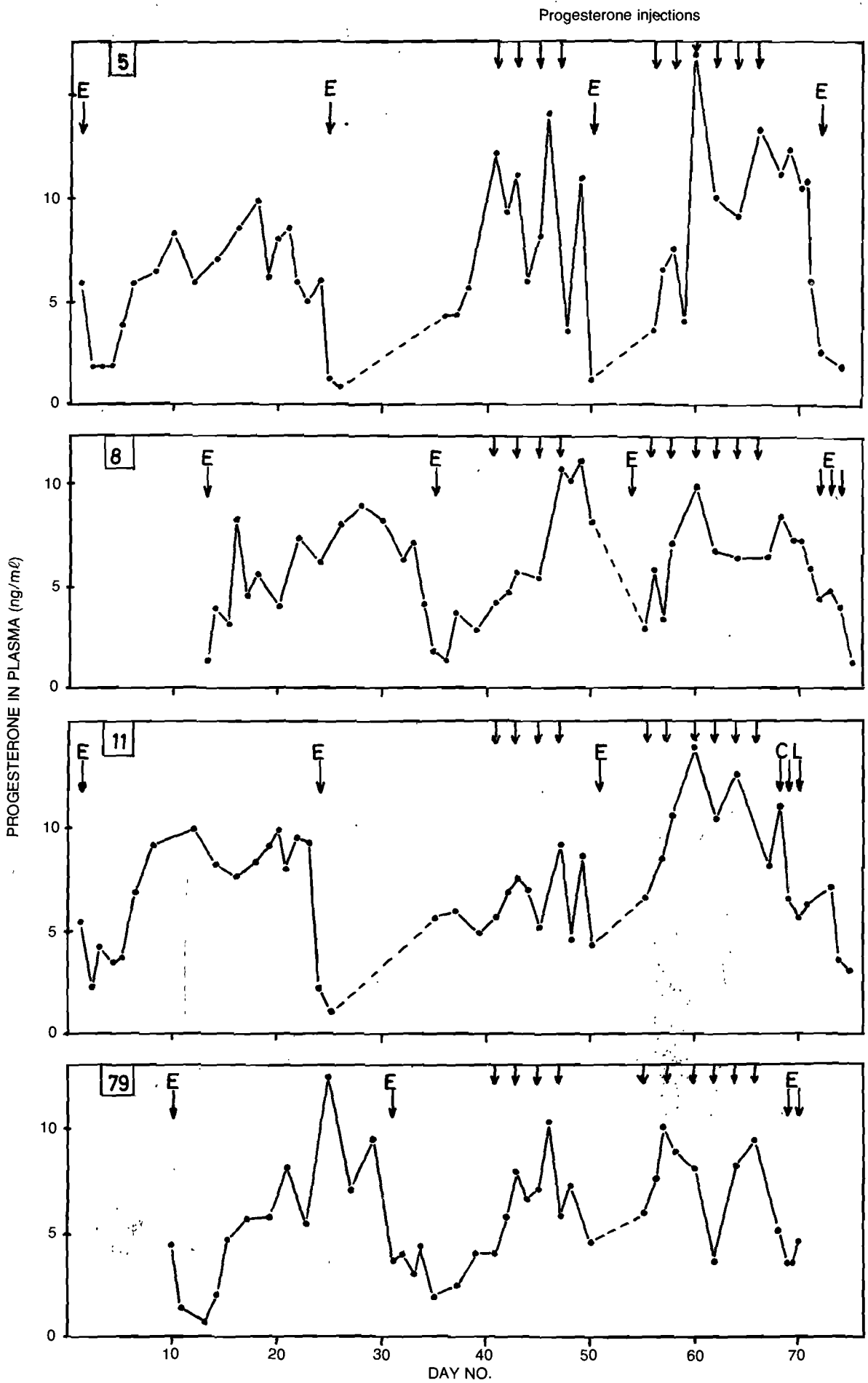

Fig. 2. Progesterone levels in the plasma of Cows 5, 8, 11 and 79 before, during and after progesterone treatment (E = Oestrus; CL = Corpus luteum).

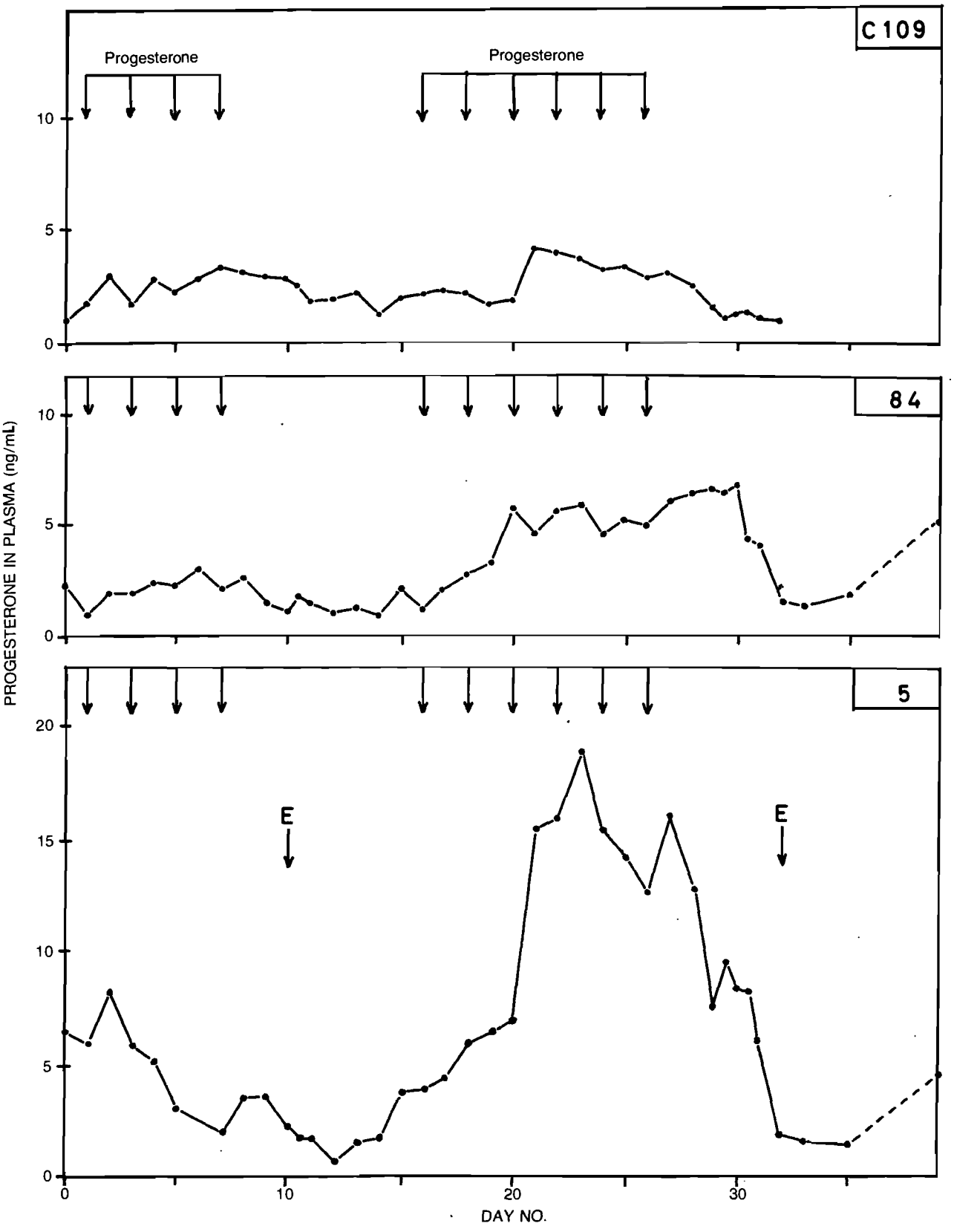

Fig. 3. Progesterone levels in the plasma of Cow C109 and heifers 84 and 5 during and after progesterone treatment (E = Oestrus)

During the periods of treatment the progesterone levels rose to approximately the same levels as during the luteal phase of the pretreatment cycles. The highest peak encountered during treatment was 17,2 ng progesterone per mℓ of plasma. The levels fluctuated considerably during the periods of progesterone administration and the peaks were not always related to a certain period after the injections. It is, however, evident that satisfactory progesterone levels could be maintained by the dosage rate and method of administration.

In three out of the four cows in trial no 1 the plasma progesterone levels did not drop as rapidly as desired after the last injection of progesterone with the result

that the onset of oestrus was also delayed. The corpus luteum of ovulation during the rest period between the two courses of progesterone administration could have contributed to this gradual decline of the progesterone levels. (See Cows 5, 8 and 11 as compared with Cow 79, Fig. 2). This suggests that either the administration of progesterone should be maintained over a longer period or a luteolytic agent such as oestradiol[18] or prostaglandin[11] [14] could be administered to exclude such corpora lutea from interfering during the anticipated synchronized oestrus period.

The three graphs in Fig. 3 show very little resemblance. The lactating anoestrous cow hardly showed any response to the progesterone injections. She was very nervous and even attempted to bite when being restrained. This lack of response to progesterone injection has been observed in other nervous cows too and gave rise to a presumption that the administered progesterone could either be utilized for corticosteroid production or else that it might be excreted more rapidly in such nervous animals.

The two heifers did not respond satisfactorily to the first course of progesterone injections but during the second course the plasma progesterone in both were maintained at or above the desired level. As judged by the subsequent rise in the progesterone levels, both heifers ovulated during the anticipated synchronized oestrous period although only heifer no 5 exhibited visible signs of heat.

CONCLUSIONS

The method for the assay of progesterone in plasma, described above, is sensitive and accurate. It can be improved further by subjecting the progesterone standards to the same ether extraction procedure as described for the samples.

Plasma levels of progesterone simulating those of the luteal phase (more than 5 ng per mℓ) can be maintained in cycling cows by injecting them intramuscularly at 48-hour intervals with progesterone in propylene glycol at dosage rates varying between 0,082 mg and 0,1 mg per kg liveweight.

A non-cycling, very nervous cow did not satisfactorily respond to the progesterone injections. Her plasma progesterone levels remained below 5 ng per mℓ throughout the period of treatment.

The plasma progesterone levels of the cows and heifers did not in all cases drop as rapidly as desired after the last progesterone injection. It appears as if the corpus luteum of the ovulation between the two courses of progesterone treatment was still active at the end of the treatment period in some of the cows and might have contributed to this gradual decline.

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THEILERIA VELIFERA DEMONSTRATED IN CATTLE IN THE EASTERN CAPE PROVINCE OF THE REPUBLIC OF SOUTH AFRICA

J. BERGER

ABSTRACT: Berger J. *Theileria velifera* **demonstrated in cattle in the Eastern Cape Province of the Republic of South Africa.** *Journal of the South African Veterinary Association* (1979) **50** No. 1, 45 (En) Kwanyanga Res Stn, Coopers (S Af) (Pty) Ltd, P O Box 5034, 5208 Greenfields, Cape Province, Rep. of South Africa.

A brief review of publications dealing with *Theileria velifera* is followed by a first record of the presence of this piroplasm in blood films from steers in the Eastern Cape Province.

INTRODUCTION

The haemoprotozoon parasite *Haematoxenus veliferus* was first reported and described in 1964 by Uilenberg from Madagascar (now the Republic of Malagasy). The organism was recorded in the erythrocytes of a calf following splenectomy and subsequently it was successfully transmitted to both splenectomised and normal cattle[8 9]. Once having recognised it as a distinct theilerial entity, bearing in some of its forms an identifying velum or veil, positive blood smears were soon obtained from cattle in several districts of the island. In the absence of clinical symptoms and since parasitaemias were consistently low, 4 % being the highest recorded at that time, *H. veliferus* was considered to be non-pathogenic. Although subsequent observations have not disproved this opinion, Uilenberg and Schreuder[13] in recording a 10 % parasitaemia in a non-splenectomised zebu have pointed out that the promotion of anaemia cannot be excluded. First attempts to transmit the organism after short term storage in liquid nitrogen failed[8], as did transmission either to a sheep subsequently splenectomised and re-challenged[8] or to a splenectomised goat[10]. The course of parasitaemia was unaffected by arsenicals, piroplasmicides or antibiotics[10]. At this stage there was already evidence that there was no cross-premunition with *Theileria mutans*[10].

Subsequently *H. veliferus* has been reported from an increasing number of localities on the African mainland, in both domestic and wild bovids and other wild ruminants. In cattle, Folkers and Kuil[3] recognised the parasite in Nigeria and Buys *et al*[1] in Uganda and Kenya. Folkers *et al*[4] found an 18 % incidence in blood smears taken from 656 White Fulani in Nigeria. Uilenberg[11] in confirming its presence in cattle in both the Central African Republic and in Chad also found 5 of 49 buffalo *(Syncerus caffer)* infected. Infection of buffalo in Uganda was also confirmed by Young *et al*[16]. Oteng and Odeke[7] examined blood slides from 10 of the poorer conditioned cattle in each of 58 herds in two different areas of Uganda and found an 18 % and 23 % incidence respectively.

Additional records in wild ruminants were forthcoming from Irvin *et al*[5] who, in a haemoprotozoon survey in the Serengeti in Tanzania, found a 2,3 % incidence of *H. veliferus* in impala *(Aepyceros melampus)* compared to a 44,7 % incidence of other theilerial organisms. Young *et al*[17] found theilerial organisms in the erythrocytes of 19 buffalo examined in the Mara district of Kenya, 12 of which were positive for *H. veliferus*. Young, in a personal communication to Uilenberg and Andreasen[12], also recorded *H. veliferus* in hartebeest *(Alcelaphus buselaphus)*.

More recently, Uilenberg and Andreasen[12] described organisms in the blood of a splenectomised sheep in Tanzania in which the velum was not contiguous with the theilerial body and they designated this piroplasm as *Haematoxenus separatus*. Uilenberg and Schreuder[13] were successful in transmitting this organism transtadially through the tick *Rhipicephalus evertsi* but failed with *Amblyomma variegatum* and *Rhipicephalus appendiculatus*. On the other hand, they were able to transmit *H. veliferus* in cattle through *A. variegatum* transtadially from nymph to adult[14]. High parasitaemias of 15 % and 10 % were recorded in a splenectomised and in a normal steer respectively.

The fact that some forms of this piroplasm, as seen in standard Giemsa-strained blood smears, are indistinguishable from those seen in other theilerial infections, has been a source of speculation as to its true relationship to other theilerial infections and in particular to *T. mutans*. Mpangala *et al*[6], using the indirect fluorescent antibody technique, have shown, however, that there is no cross-reaction between *H. veliferus* and *T. mutans* or *T. parva*.

A recent study of *H. veliferus* by both light and electron microscopy carried out by Van Vorstenbosch, Uilenberg and van Dijk[15] has shown that the ultrastructure of the oganism is similar to that of other *Theileria* species, that the veil has a crystalline or paracrystalline structure and there there is a uniformally thick gap of apparently normal red cell cytoplasm between the organism and the veil. In discussing these findings and relating them to the characteristics of veil-like structures reported in some *Theileria* from both cattle and wild ruminants, these workers have now discarded the generic name *Haematoxenus* and re-named the two species *Theileria velifera* and *Theileria separata*. This nomenclature is adopted in the ensuing text of this report.

HISTORY

In the absence of any published confirmation of the occurrence of *T. velifera* in the Republic of South Africa it is pertinent to record finding this piroplasm in blood slides from yearling steers on the farm Gulu near East London. Stock on this farm are allowed to become tick-infested to facilitate the conduct of acaricide trials and heavy infestations of *Boophilus decoloratus, R. evertsi, R. appendiculatus* and *A. hebraeum* are not uncommon.

Steer 1, having failed to maintain improvement in condition after imidocarb dipropionate* treatment for a clinical episode due to *Babesia bigemina,* was kept at the laboratory for observation. This steer exhibited an

*Imizol. Burroughs Wellcome & Co., The Wellcome Foundation Ltd., London and Berkhamsted.

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obvious *T. velifera* parasitaemia, varying between 0,1 % and 1 % over a period of 10 weeks, with a high proportion of organisms placed peripherally in the erythrocytes and possessing a well marked velum standing out from the erythrocytic perimeter, giving these cells a "split-open" appearance. At the time that *T. velifera* was first seen in this steer, blood slides, taken at autopsy from Steer 2 which died of snakebite, also proved positive for *T. velifera.* Subsequently slides have proved positive from many other steers in this herd.

Heparinised blood (16 mℓ) from Steer 1 was given by the intravenous route to each of two yearling splenectomised steers. It was hoped that Steer 1 might have been free of *B. bigemina* following a potentialy sterilising dose of imidocarb dipropionate. Nevertheless, both splenectomised steers, which had previously been artificially infected with *B. bovis* and had also suffered primary and relapse *Anaplasma marginale* parasitaemias, exhibited a *B. bigemina* reaction 5 days later. Treatment with 3 mg/kg imidocarb dipropionate rapidly cleared this infection and first *Theileria* spp. piroplasms were seen 19 days after blood challenge in one splenectomised steer only. The following day, after prolonged search, one erythrocyte with a typical *T. velifera* organism bearing a veil was recorded. Although the number of piroplasms increased steadily to reach a 50 % parasitaemia, no more typical forms were observed up to six weeks after attempted transmission. The other splenectomised steer was sent to the abattoir 21 days after challenge, by which time no theilerial organisms had been recorded in blood smears.

DISCUSSION

Although study of the organism and further attempts at transmission must be postponed owing to other priorities it is hoped that this short review and record may prompt local interest. If *Amblyomma* spp. is the vector, as seems likely, then it seems equally likely that *T. velifera* will prove to have a wider distribution in South Africa than appears at present; although the importance of wild ruminant carriers has not been established, this source is also well provided. de Vos[2] (personal communication) has recognised the organism in slides from Transvaal cattle submitted to Onderstepoort.

In scrutinizing this organism for the first time one is tempted to look back in retrospect and wonder how often one has missed it in the past by considering it an artefact resulting from faulty slide preparation or staining. A perusal of the excellent illustrations made by Uilenberg[9] may act as a stimulus towards a more careful scrutiny of the many hundreds of blood slides examined daily in areas where tick borne disease is common.

Concurrent haemoprotozoon infections and the necessity for chemotherapy with imidocarb dipropionate may have contributed to the failure of this single attempt to establish the infection in the two splenectomised steers.

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THE SAFETY OF FENTHION 20 % m/v* WHEN APPLIED TOPICALLY TO PREGNANT COWS

J.P.J. JOUBERT and J.A. MINNE

ABSTRACT: Joubert, J.P.J.; Minne, J.A. **The safety of fenthion 20 % m/v when applied topically to pregnant cows.** *Journal of the South African Veterinary Association* (1979) **50** No 1 47 (En) Toxicology Section, Veterinary Research Institute, 0110 Onderstepoort, Republic of South Africa.

A pilot trial was conducted with fenthion 20 % m/v to determine possible abortifacient or other toxic effects when applied to 4½ month pregnant beef cows. No abortions or other clinical symptoms which could be attributed to the fenthion were observed after two topical applications, at a 3-week interval, of up to 5 times the therapeutic dose.

INTRODUCTION

A complaint from a farmer regarding the possible abortifacient effect of fenthion 20 % m/v in cows motivated this trial. Fenthion is normally applied topically to the back and dorsal lumbar region of cattle to control louse infestations and its recommended dosage regimen is 2 applications with a 3-week interval between treatments. Like all organophosphorus compounds it owes it's toxicity and action to the inhibition of the enzyme acetylcholine-esterase. The use or misuse of this group of compounds is not usually associated with abortions[1].

MATERIALS AND METHODS

Half a litre of fenthion 20 % m/v (batch No. TSM4) was received from the farmer concerned.

Six crossbred beef-type cows previously inseminated were selected for the experiment. Pregnancy of ≤ 4,5 months was confirmed by rectal examination at the outset of the experiment. The body mass of each cow was determined and the dosages of fenthion 20 % m/v used (see Table 1) calculated on that basis. The cows were kept in paddocks where they were observed daily throughout the trial period of 6 weeks whereafter they were returned to natural grazing. Calving dates were obtained from the herd manager at a later stage.

The fenthion was poured onto the dorsal flat surface of the lumbar area of the experimental animals. Each cow received 2 applications at an interval of 3 weeks.

Table 1: DOSAGE REGIMEN

Cow No.	Body mass (kg)	Multiples of Therapeutic dose*	Total dose (mℓ)
1	508	1,97	25
2	545	2,86	39
3	451	2,84	32
4	382	4,40	42
5	444	4,95	55

*Therapeutic dose = 2,5 mℓ/100 kg body mass.

The thin layer chromatography method used to determine the composition of the specimen of fenthion 20 % m/v, was as follows:

Two microlitres of a 0,1 % solution of the specimen, in chloroform was spotted on silica gel GF 254 plates. Standards of fenthion and ethyl fenthion were spotted on the same plate for comparison. Two mobile phases were used, namely, benzine and a hexane acetone mixture (8:2).

Whole blood choline-esterase (Che) values were regularly determined on all the cows, each animal serving as its own control. The following modification of the method of Ellman, Courtney, Andres and Featherstone[2] was used: Heparinised blood was haemolysed with 9 volumes of distilled water and the haemolysate immediately diluted to 1:1000 with 99 volumes of 0,1M phosphate buffer, pH 8,0. Of this solution, 3,5 ml was heated to 37 °C in a waterbath and the chromogen, consisting of 25 µl of 0,1 M dithiobisnitrobenzoic acid (Eastman Kodak) in 0,1 M phosphate buffer, pH 7, added. The substrate consisting of 25 µl of acetylthiocholine (Eastman Kodak) 0,1 M in distilled water was then added. The change in optical density (ΔOD) at 410 nm over a 5 minute period in relation to a blank, containing all the ingredients except the substrate, was then determined. The value for spontaneous hydrolysis was also obtained and deducted from the ΔOD.

RESULTS AND DISCUSSION

The specimen of fenthion 20 % m/v under suspicion was shown to be identical with the commercially available product by thin layer chromatography. The Rf values for fenthion and ethyl-fenthion in benzine were respectively 0,6 and 0,65. In hexane acetone the values were 0,4 and 0,5 respectively. Quenched spots were observed under UV light and, alternatively, the plates were brominated before spraying with a fluorescein solution in alcohol (96 %) and observed under UV light (254 nm).

Che values of 10–20 absorption units are regarded as normal, 5–10 absorption units as slightly depressed, and 0–5 absorption units as well depressed. Table 2 shows that Che values were slightly depressed in cow 5, but within normal values in the other animals.

No clinical symptoms of toxicity or abortions were observed during the trial period of 6 weeks, after which the animals were discharged from the experiment. They all calved normally approximately 3 months later, with the exception of Cow 3, which aborted about a week before full term and died 11 days later. Unfortunately the cause of death could not be determined on account of advanced *post mortem* changes. It seems reasonable to assume, however, that the abortion and death was unrelated to the application of fenthion 20 % m/v, 3 months earlier.

*Tiguvon Spot On, Bayer South Africa (Pty) Ltd.

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Table 2: CHOLINE-ESTERASE (CHE) VALUES

Choline-esterase values (absorption units)					Time determined
Cow No.					
1	2	3	4	5	
c2x therap. dose	c3x therap. dose	c3x therap. dose	c4x therap. dose	c5x therap. dose	
16,5	16,5	16,5	18,0	18,0	immediately before 1st treatment
15,5	13,0	14,5	15,0	16,0	2 h after 1st treatment
15,5	16,5	15,5	17,5	15,5	24 h after 1st treatment
14,5	15,5	14,5	15,5	13,5	4 days after 1st treatment
14,0	14,5	12,5	13,5	12,5	7 days after 1st treatment
14,0	15,0	12,5	14,5	12,0	14 days after 1st treatment
13,0	13,5	12,5	13,5	11,5	21 days after 1st treatment
13,0	14,5	14,0	14,0	12,5	24 h after 2nd treatment
15,0	15,0	13,0	12,5	10,0	28 h after 2nd treatment
14,0	13,5	12,5	12,0	10,8	4 days after 2nd treatment
12,5	14,0	12,0	11,0	8,5	7 days after 2nd treatment

ACKNOWLEDGEMENT

The authors wish to thank the Director, Veterinary Research Institute, Onderstepoort, for permission to publish this article. A special word of thanks is due to Dr T.S. Kellerman and the staff of the Toxicology section.

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BOOK REVIEW — BOEKRESENSIE

KRANKHEITEN DES RINDES

G. ROSENBERGER 2nd Ed.
Verlag Paul Parey, Berlin & Hamburg 1978
pp. XXVII 1403 Figs 747 (28 colour) Tabs 70, Publ. Price DM 390

The first edition of Krankheiten des Rindes was published in 1970. Apart from updating the therapeutic index – which can also be bought separately – the main text remains unaltered for the 1978 2nd edition.

The unchanged text is an indication of the initial comprehensiveness of the contents of this remarkable book which is distilled from more than forty years experience of Professor Rosenberger and his three collaborators in the bovine clinics at the veterinary schools of Hannover, München and Giessen.

The text covers sporadic and related specific diseases of organs and organ systems as well as infectious, parasitic, metabolic and deficiency diseases. Toxicology, therapy and surgical procedures are discussed when applicable.

The well selected bibliography represents publications up to 1970 only. In a new edition one obviously would have liked to see the addition of more recent work. On rereading the text however I was again impressed with the fact that the first edition was so well prepared that only significant improvements in therapy could be added and this was indeed accomplished by modernising the therapeutic index in the second edition.

Krankheiten des Rindes is the most comprehensive volume on bovine diseases available in the world veterinary literature. It is less than likely that this massive work will be translated into the English language. The contents will therefore only be accessable to readers with a knowledge of German. On the other hand the photographs, illustrations and tables alone are sufficiently valuable for the non-German reader.

For the German-reading veterinarian who has, in whatever capacity, something to do with cattle, Krankheiten des Rindes will remain the most consulted book in his library no matter how extensive the latter is.

As can be expected from Verlag Paul Parey the printing, binding and finish of the volume is beyond criticism.

K.v.d.W.

CASE REPORT **GEVALVERSLAG**

BOVINE CEREBRAL THEILERIOSIS (TURNING SICKNESS) WITH SPINAL CORD INVOLVEMENT

R.C. TUSTIN* and J. VAN HEERDEN†

ABSTRACT: Tustin R.C.; van Heerden J. **Bovine cerebral theileriosis (turning sickness) with spinal cord involvement.** *Journal of the South African Veterinary Association* (1978) **50** No 1, 49 (En) Dept. Pathology, Faculty of Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Rep. of South Africa.

A case of bovine cerebral theileriosis is described in which the principle lesions occurred in the spinal cord and its meninges. These were clogging of vessels with lymphocytes, many of which were parasitised by *Theileria* sp. schizonts (probably *T. mutans*), thrombosis and infarction of nervous tissue, and lymphocytic meningomyelitis. Rare cerebral meningeal vessels showed thrombosis. Clinically the animal showed posterior paresis.

INTRODUCTION

Bovine cerebral theileriosis (turning sickness or *draaisiekte*) is an acute, subacute or chronic, sporadic, afebrile disease in cattle which is characterized clinically by the development of nervous signs including one or more of the following: circling or turning, epileptiform fits, depression, paralysis, paresis and ataxia[2 4 6 7]. Pathologically clogging or plugging is evident of blood vessels of the central nervous system and meninges by emboli comprising cells of the lymphocytic series, many of which are parasitised by schizonts of the genus *Theileria,* with associated thrombosis, infarction and haemorrhage, and lymphocytic meningo-encephalitis.

In the Republic of South Africa it is considered that the disease is probably one of the manifestations (albeit rare) of infection with the protozoan parasite, *Theileria mutans*[2 4 6 7] but in other parts of Africa where the condition has occurred *Theileria parva*[4], which was eradicated from South Africa in 1954[5], has also been implicated. The pathogenesis of the condition, however, remains enigmatical; one possibility is that an autoimmune disorder, induced by the parasite within lymphocytes, is responsible for the intravascular agglutination of lymphocytes[7] with subsequent embolism, superimposed thrombosis, and infarction.

Sporadic cases of turning sickness occur in cattle in certain areas of this country where infection with *T. mutans* is prevalent. Its symptomatology and pathology have been well documented[2 4 6 7], but it is only in one article that brief mention of spinal cord involvement of affected animals is made[6]. As no details are given, the particular case under review in which the main lesions occurred in the spinal cord, was deemed worthy of note.

HISTORY AND CLINICAL SIGNS

The animal, an 11 month-old cross Africander castrated male, was born and reared on a farm in the Rietgat area of the Pretoria district. When presented for examination during April 1978 it showed complete paralysis of the hindquarters. According to the owner, the animal was paretic on the previous day.

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No fever was present and the animal's "psyche" was fairly bright. Normal forelimb, cranial and panniculus reflexes were present. The flexor reflex was present in the right hind limb but absent in the left hind limb. The tail was insensitive to pinpricking and showed flaccid paralysis. Appetite was depressed and no ruminal movement could be detected.

Cerebrospinal fluid which was collected from the lumbosacral site was found to be high in protein (Table 1).

Unfortunately no blood smears were examined.

As the prognosis was considered to be extremely grave, it was destroyed by the intravenous administration of an overdosage of a pentobarbitone sodium solution. The owner of the animal reported that 4 other cattle had died on his farm during the previous 2 months. All had shown nervous symptoms, viz. paresis or paralysis but none had been examined by a veterinarian

Table 1: COMPOSITION OF CEREBROSPINAL FLUID COMPARED TO NORMAL BOVINE VALUES AND THOSE IN OTHER CASES OF TURNING SICKNESS

	Normal values[3]	Patient	Values in the cases of turning sickness of Carmichael & Jones[1]
Colour	Clear	Clear	Clear-turbid
S.G.	1,006–1,007	1,007	–
Protein g/ℓ	0,2–0,33	0,96	0,2–0,6
Glucose m mol/ℓ	1,94–3,89	3,02	1,19–2,95
White Cell Count cells/mm³	0–10	1	1–36

PATHOLOGY

Gross pathology

With the exception of changes associated with pentobarbitone sodium poisoning, lesions which were considered to be of significance were found only in the central nervous system. On removal of the *spinal cord* from the vertebral cord it was noticed that a segment of its mid-thoracic portion about 50 mm long showed slight swelling, i.e. an increase in diameter. This was more apparent by palpation than visually. On transverse sec-

0038-2809/79/01/0049

tioning of the middle of the affected part, it was apparent that the whole of the grey matter and the majority of the white had a light brown discolouration and that the cut surface was slightly oedematous. The discoloured part was softer than normal.

The whole of the spinal cord with its dura mater largely intact was fixed in 10% formalsaline for subsequent more detailed macroscopic, and for histopathological examination. Gross examination of numerous serial coronal sections of the swollen spinal cord revealed that the extent of the discoloured part gradually tapered to its cranial and caudal limits and that the grey matter was more severely affected than the white. Numerous additional coronal sections of the rest of the spinal cord were made and the incised surfaces examined; no lesions were encountered.

In 4 relatively large blood vessels of the pia mater covering the dorsal aspect of the *cerebrum* thrombi were present. Short segments, about 3–4 mm long, of the vessels were affected. The thrombi were a dull yellow-brown and had caused vascular distension. Serial coronal sections of the whole brain about 4–5 mm in thickness were cut and examined but no lesions were observed. No apparent changes in nervous tissue were associated with the meningeal thrombi.

Specimens of the affected meninges and underlying cerebral tissue and of representative areas of other parts of the brain were fixed in 10% formalsaline for histological study.

Histopathology

Numerous sections from representative parts (cervical, thoracic, lumbar and sacral) of the spinal cord, where possible with the associated dura mater and arachnoid, and the brain were cut and stained with haematoxylin and eosin, Giemsa and by Perl's method for iron.

In the macroscopically affected part of the thoracic *spinal cord* very severe pathological changes were present. These resembled those which have been described in the brain[2 4 6 7], and its meninges and comprised the following: "Plugging or clogging" of numerous capillaries and larger blood vessels in the nervous tissue and pia mater and arachnoid by lymphocytes many of which contained intracytoplasmic schizonts of the genus *Theileria* (or Koch's bodies). A relatively high mitotic index in these cells was apparent. Extracellular schizonts were also present. Many of the other vessels in the pia mater and arachnoid and nervous tissue contained thrombi, the majority being of the occluding type. In some vessels thrombi and lymphocytic plugs were present. In the most severely affected part of the spinal cord, the whole of the grey matter and a large portion of the white matter showed extensive changes. The entire grey matter was encephalomalacic and glial cell proliferation was prominent. No normal neurons could be found. There was proliferation of capillaries which was made more apparent by the fact that many of them contained, and were distended, by plugs of lymphocytes or thrombi. The white matter showed large areas of demyelination which, in parts, had resulted in microcavitation. In association with these lesions were scattered very small fresh haemorrhages and evidence of past haemorrhage, viz. the presence of haemosiderin-laden macrophages. Oedema was also present.

A mild infiltration of lymphocytes was present in the pia mater and arachnoid and several vessels in the nervous tissue exhibited perivascular lymphocytic cuffing.

The spinal dura mater in the area where the cord was extensively damaged only showed a mild perivascular lymphocytic cuffing of a small number of its blood vessels. It is interesting to note that in this area of the cord thrombosis and lymphocytic clogging of some vessels, congestion and rare small haemorrhages were observed in several of the roots of spinal nerves. In addition a small number showed demyelination.

In the rest of the spinal cord and its related structures, which were grossly normal, very few changes were observed. These comprised rare mild lymphocytic cuffs around blood vessels of the spinal cord, dura mater and epineurium of the roots of spinal nerves (especially in the cauda equina) and very small haemorrhages in the nervous tissue of the cord.

Microscopic *cerebral lesions* were confined chiefly to the grossly affected part of the pia mater and arachnoid and very closely resembled those of the spinal meninges. Apart from the presence of a mild degree of lymphocytic cuffing around a small number of blood vessels, no other lesions of significance were encountered.

DISCUSSION

Most of the cases of turning sickness described by previous authors[2 4 6 7] have occurred in young to young adult cattle; the age of the animal described here was 11 months. As in the cases of van Rensburg (1976)[7] it occurred in the late summer, at a time when tick infestation is high. This might possibly play a role in the incidence of the disease[4 7]. However, unlike the 5 cases described by van Rensburg no focal infarcts associated with thrombosis or the clogging of vessels with lymphocytes were noticed in the spleen.

As far as it is known this is the first case which has been reported in which lesions, and the principle ones at that, have been described in the spinal cord although brief mention of spinal cord involvement was made by Schulz and Schutte (1957)[6]. The most important lesions in our case occurred in the thoracic spinal cord and its meninges; those in the cerebral meninges were less significant. However, none of these pathological changes were macroscopically prominent and could easily have been overlooked.

The lesions in or around blood vessels in the spinal dura mater and some spinal nerve roots is of interst and warrants more detailed investigation in future cases of turning sickness, as indeed does involvement not only of the spinal cord, but also of the cranial and peripheral nerves and ganglia.

Although Carmichael & Jones (1939)[1] used the atlanto-occipital site for the collection of cerebrospinal fluid in their studies on the changes in the composition of this fluid in bovine cerebral theileriosis, we preferred the lumbosacral because it is technically easier. However, the only abnormal finding was an elevated total protein value in contrast to their cases which showed in addition, in some cases, turbidity, amber discoloration and increased white cell count.

The most important clinical differential diagnosis to our case of turning sickness is perhaps a sporadically occurring condition in cattle colloquially known as *"sitsiekte"* in Afrikaans (or "sitting sickness") in which the presence of an epidural abscess, most frequently caused by *Corynebacterium pyogenes,* exerts pressure on the

spinal cord and causes the animal to assume a position of a sitting dog due to posterior paresis or paralysis. Its incidence has also been related to heavy tick infestation and it occurs particularly in areas where *T. mutans* may be expected.

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We are indebted to Mrs V.K. Käber for typing the manuscript.

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Op die oorkantse bladsy verskyn 'n klasfoto van die Finalejaar studente van 1978. Tydens 'n gradeplegtigheid op 17 November 1978 is die BVSc-graad aan 35 nuwe veeartse toegeken.

Na voldoening aan al die vereistes vir die graad kan die ander kandidate ook verwag om in die eerskomende maande te gradueer. **Die SAVV verwelkom hulle almal as lede van die professie.**

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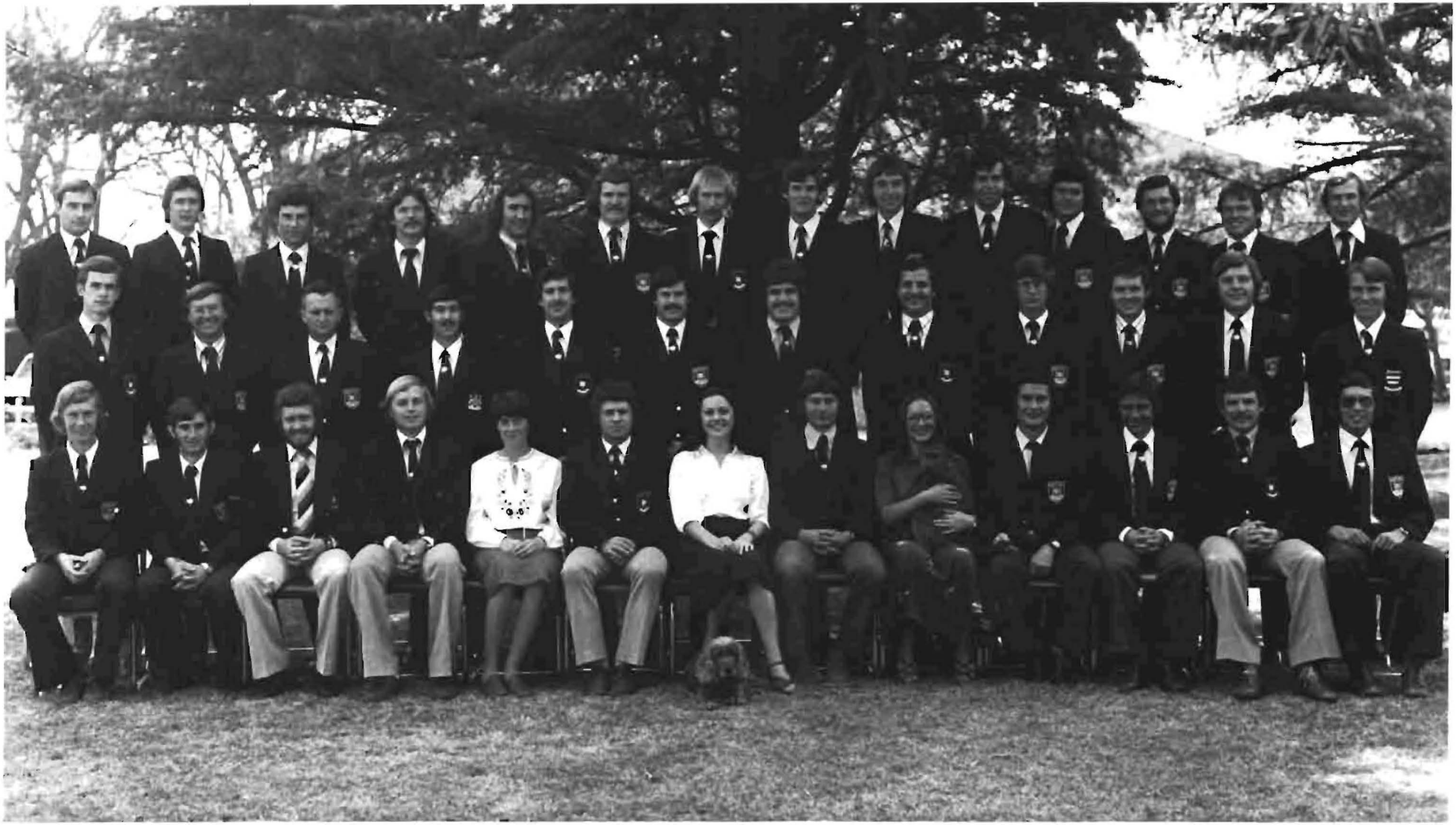

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Meer as 130 jaar lank al wen Coopers die stryd teen uitwendige parasiete!

Vandag is ons trots dat ons die boer 'n volledige reeks skaapdippe kan bied om aan alle vereistes te voldoen insluitende:

Lindaan "FLO" Brandsiektedip. LINDAAN of ϒ -BHC is 'n hoogs doeltreffende verbinding wat die gevreesde brandsiektemyt wat so baie probleme en finansiële verliese teweegbring, dood. Twee (2) dippings met 'n tussenpose van 7 — 10 dae word onder Staatstoesig vir die uitroei van die parasiete vereis. Hierdie diproetine word ook deur die wet vir die beheer van bokskurfte vereis.

Lindaan "FLO" Brandsiektedip dood ook luise en skaapluisvlieë en beheer Karooverlammingsbosluise.

Diazinon D.F.F. 'n Hoogs gekonsentreerde oplosmiddelvrye dip wat skaapbrandsiektemyte, bokluise, skaapluisvlieë en bosluise dood en beskerming teen brommeraanvalle verleen. Soos in die geval met die ander Coopers D.F.F. dippe word die risiko van velbrand "droë punte" wat by ander dippe voorkom, verminder as gevolg van die afwesigheid van oplosmiddels.

Goue Vag D.F.F. Die een-skoot, hoogs gekonsentreerde oplosmiddelvrye skaap- en bokdip wat jeukmyt, alle bosluise insluitende Karooverlammingsbosluise, luise en skaapluisvlieë dood en teen brommeraanvalle beskerm.

Supadip D.F.F. 'n Oplosmiddelvrye, hoogs gekonsentreerde skaap- en bokpensbaddip vir gebruik met die Voortdurende Aanvullingsmetode om bosluise, insluitend die Karooverlammingsbosluise, luise en skaapluisvlieë te beheer en teen brommeraanvalle te beskerm. Die Voortdurende Aanvullingsmetode as dit by 'n pensbad gebruik word, sal altyd die dipvlak op 'n konstante hoogte hou terwyl 'n eenvormige neerslag aktiewe bestanddeel op elke dier verseker word. Dit bring mee dat daar 'n aanhoudende hoë biologiese doeltreffendheid verkry word met geen gevaar van vermorsing of oorbehandeling nie.

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