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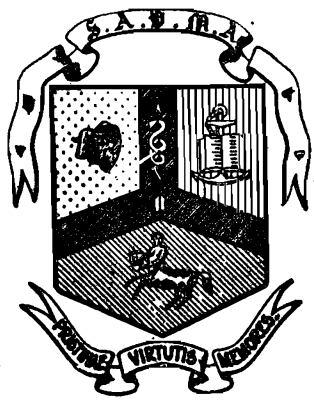

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VAN

DIE SUID-AFRIKAANSE VETERINÊR-MEDIESE VERENIGING

LIVE WEIGHT LOSS IN SLAUGHTER CATTLE SUBJECTED TO PROLONGED RAIL TRANSPORT WITHOUT FOOD, WATER AND REST.

L.W. VAN DEN HEEVER* and G. D. SUTTON**.

SUMMARY

A truckload of mature slaughter steers, sent on an unbroken train journey of four days without off-loading for food, water or rest in midsummer in the Republic of South Africa under hot and severe climatic conditions, experienced a mean loss of 19.7% of their live weight. Loss of ingesta accounted for only 7.0%, the remaining 12.8% being lost in body tissues and fluids. The pH of muscle 24 hours after slaughter was normal, but mean moisture content was decreased by 2.2%. The resultant overall decrease in carcase yield is considered sufficiently significant to make such a method of transport impracticable, apart from obvious humane considerations. Statistical analysis of data indicates the sources of live weight loss.

INTRODUCTION

During an earlier investigation into the feasibility of transporting slaughter cattle by rail over long distance without the usual periodic 4-hour interruptions for feeding, rest and water[1], the impression was obtained that the loss in live weight during 4 days of travel could only partially be explained by reduction of ingesta. A controlled study was therefore undertaken to verify this impression and to obtain reliable data of weight loss incurred by the different body components.

EXPERIMENTAL PROCEDURE

Thirty five similar mature dehorned Afrikaner type steers were divided into two groups. The control group contained 15 animals and the experimental group of 20 was railed. Prior to the experiment both groups were kept together in a large enclosure for 10 days. Water, veld and lucerne hay were available *ad lib.*

All animals were weighed and the control group was slaughtered. After elimination of an injured animal from the experimental group, the remaining 19 were loaded into a GZ-type railway truck, 10 animals in one compartment and 9 in the other. They travelled for 96 hours on rail in the northern and north-eastern Transvaal without being detrained for food, water or rest. Conditions were extremely hot, dry and trying, as exemplified by the data, for a town in the area, supplied by the Climatology Section of the Weather Bureau. (Table 1).

TABLE 1.—CLIMATOLOGICAL DATA AT POTGIETERSRUST DURING EXPERIMENTAL TRANSPORT.

	Temperature °C		% Relative Humidity	
Day	Max.	Min.	8 a.m.	2 p.m.
1	36.6	16.2	31	9
2	35.0	21.3	46	14
3	36.0	21.3	30	9
4	36.5	22.0	19	9

On return, one steer was found injured in the truck; therefore, data from it were not used. The remaining 18 animals were immediately offloaded, weighed and slaughtered without being given access to food and water. The weights of the live animals, warm carcases, ingesta, viscera, etc. of the animals of both groups were recorded. "Blood etc." included miscellaneous trimmings lost during slaughter and dressing. Data are furnished in Table 2.

After the carcases had been stored at −2°C for 24 hours, the moisture content and pH of portions of the *M. triceps brachii* were determined, the former electrometrically and the moisture content by drying a sample of known weight at 100°C to constant weight. These figures are given in Table 3.

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TABLE 2.—SUMMARY OF LIVE WEIGHTS (LB) AND SLAUGHTER DATA OF CONTROL AND EXPERIMENTAL STEERS.

	Statistical Data					
	Mean		Std. deviation		Adjusted* mean	
	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.
Live weights:						
Initial	1267.1	1271.2	93.7	90.6	1269.5	1269.5
After Rail	—	1019.7	—	75.1	—	1018.3
Loss	—	251.6	—	21.4	—	251.2
Warm Carcase:						
Weight	697.1	610.0	71.7	56.4	698.4	609
% of Initial	54.9	47.9	2.1	1.9		
Grade	II—	II—	—	—	—	—
Digestive Tract:						
Full	265.0	163.8	20.0	19.7	265.4	163.7
Empty	75.6	63.3	6.27	4.9	75.7	63.2
Ingesta	189.4	100.5	17.0	18.4	189.7	100.5
Other offal	204.8	190.6	13.1	12.4	204.8	190.5
Blood etc	100.8	55.2	14.5	10.4	100.9	55.1

*Adjusted for regression.

DEDUCTIONS

1. By covariance analyses with initial weight as concomitant variable it was found that in all instances the differences in the component weights of the experimental and control animals were significant ($P < 0.005$).
2. The adjusted mean ingesta weight of the 15 control animals (A) amounted to 189.7 lb or 14.9% of mean preslaughter live weight and 27.2% of the warm carcase weight. On the basis of similarity of groups it was estimated that the ingesta of experimental steers before travel would also have amounted to a mean of 14.9% of their live weights, i.e. 189.7 lb (B).
3. After 4 days travel the adjusted mean loss of live weight of 18 experimental steers amounted to 251.2 lb (C). This constitutes 19.8% of pretravel live weight.
4. The actual mean weight of ingesta after travel (D) was established at 100.6 lb. B-D would then provide the estimated mean weight of ingesta lost in transit, i.e. 89.2 lb (E). This loss in weight is significant ($P < 0.01$). The estimated loss of ingesta constitutes only 35.9% of actual live weight loss (C) and leaves an amount of 162.0 lb to be accounted for in some other way.
5. On the basis of group similarity, Table 2 can also be used to determine other sites of weight loss in the experimental animals:

Site:	*Weight (lb.)*	*S.E. of difference between adjusted means*:
(a) Warm Carcase	89.4	6.89
(b) Gastro-intestinal Tract	12.5	1.22
(c) Other offal	14.3	3.49
(d) Blood, etc.	45.8	4.15

As blood constituted by far the largest component of (d), it must be assumed that considerable dehydration existed. Alternatively, bleeding-out was poor, but the latter situation would be reflected in carcase weight.

6. The mean losses in live weight of mature steers after four days rail travel may be broken down as follows:

Site	*lb*	*%*
Ingesta	89.2	35.5
Carcase	89.4	35.6
Digestive Tract	12.5	5.0
Other viscera and organs, including omentum	14.3	5.7
Blood and Slaughter waste	45.8	18.2
Total	251.2	100.0

The mean reduction of carcase weight of some 89 lb due to travel constitutes a considerable financial loss. If calculated at the

average current price of R15.00/100 lb, this amounts to a direct loss of R13.35 per animal of this class, weight and grade.

TABLE 3.—THE pH AND MOISTURE CONTENT OF *M. triceps brachii* AFTER 24 HOURS AT —2°C: MEAN AND RANGE.

Group	pH	% moisture
Controls (15).....	5.9 (0.2)	76.6 (2.9)
Experimentals (18).....	5.8 (0.3)	74.4 (5.2)

DEDUCTIONS

1. The differences in pH values of meat from the two groups of animals are neither statistically nor biologically significant.
2. After executing an arc-sine transformation of the percentage moisture in the muscle specimens, the T-test was employed. The mean difference of 2.2% between experimental and control animals is significant ($P < 0.001$).
3. Based on a 60:40 proportion of muscular tissue to other tissue in the carcase (Ziegler[2]), normalisation of the moisture content of a carcase with a mean weight of 609 lb for the experimental group would only contribute 8 lb per ox.
4. Table 2 showed that the adjusted mean difference in carcase weights between the control and railed groups was 89.4 lb. If only 8 lb of this was lost as moisture from the musculature, there remains 81.4 lb lost in other ways, possibly by tissue breakdown and utilisation.

DISCUSSION

Reports on various aspects of live and carcase weight losses resulting from fasting and transport of pigs and lambs have appeared, but similar work on cattle is not known. Shier, and Callaghan and Thompson, quoted by Starke[3], reported that fat lambs lose up to 13.5% of live weight after 77 hours of fasting and 11.2% after 96 hours respectively. The same authors recorded carcase weight losses up to 4.9% after 77 hours and 5.7% after 96 hours of fasting respectively. These figures are considerably lower than those obtained in cattle in our study.

Trowbridge *et al*, quoted by Starke[3], found that external carcase tissues lose their fat more rapidly than other parts of the body, whereas Callaghan and Thompson detected no reduction in the weight of caul fat of fat lambs after 4 days fasting. Our findings do not confirm these reports when considering the general loss of carcase weight.

Concerning loss of ingesta from the digestive tract of cattle, Forbes *et al*, and also Dukes, both quoted by Starke[3], state that the bulk of the material present is eliminated within the first 2-3 days of fasting. Our present study disclosed that after four days of complete fasting in transit, cattle still retained more than 50% of their ingesta.

Starke[3] states that neither Callow nor Callaghan and Thompson detected a decrease in the blood content of pigs and fat lambs respectively after fasting. Bianca[4] found that plasma volume was reduced by 20% after dehydration of steers over 4 days, and considered that this water deficit represented some 8% of body weight. Our study on cattle weighing 1200 lb disclosed only about 4% shrinkage due to reduction of blood volume after fasting in transit over 4 days.

Bisschop and Hirzel reported to Starke[3] that no reduction in moisture content of muscle (*M. longissimus dorsi*) occurred in cattle transported over 48 hours and then rested for 18-14 hours before slaughter. Starke[3] concluded that a "large portion of loss in carcase weight" could be attributed to loss of muscle moisture. Their animals, however, had access to water, and Bianca *et al*[4] have subsequently demonstrated that most repayment of the water debt of dehydrated animals occurred within the first day of rehydration, particularly at high ambient temperatures. Our study showed that, even under extreme conditions, heavy cattle lost only about 8 lb in carcase weight due to decrease in muscle moisture content.

In general the failure of our findings to agree with those of other workers may be ascribed in part to species differences and the fact that the cattle in our studies were exposed to rather extreme conditions. Starke[3] quotes various reports indicating that shrinkage in pigs and lambs was greater during hot dry summer weather than during wet cold or temperate weather.

GENERAL CONCLUSIONS

1. Loss of weight in uninterrupted transit was not entirely due to loss of ingesta; more than 60% occurred in the carcase, viscera and blood. This represents significant economic loss to the producer in terms of actual carcase weight.
2. It appears that in addition to some slight loss of moisture content, weight loss in the carcase and viscera may be due to tissue breakdown; the latter is not likely to be readily restored during the short rest period usually given to stock prior to slaughter even when food and water is provided.

3. Dehydration of blood appears to constitute a considerable amount of live weight loss (18%) and this could well be corrected by allowing the animals access to water during a pre-slaughter period of rest.
4. Apart from humane considerations, the continuous transport of cattle by rail for four days without offloading for food, water and rest cannot be recommended in view of significant economic loss which would result.
5. The effect of interrupted rail travel under existing railway regulations on live and carcase weight losses needs to be investigated anew.

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WORLD ASSOCIATION FOR BUIATRICS

V. International Meeting on Diseases of Cattle from September 13th to 17th, 1968, in Opatija/Yougoslavia

The following topics will be considered:

Opening topic: Veterinary problems concerning large herd management.

Main topics: Diseases of new-born calves; — Mastitis; — Abdominal surgery in cattle.

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Announcements of papers to the general topics and free topics may be submitted to the Organizing Committee not later than December 31st, 1967.

Languages of the meeting are English, German and French, simultaneous translation being provided for.

PLASMA ENZYMES IN BLUETONGUE.

R. CLARK* and ADRIANA M. WAGNER*.

SUMMARY AND INTRODUCTION

In a preliminary communication[1] it was reported that the plasma levels of glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH) and aldolase (Ald) had been found to rise after the subsidence of the fever reaction in bluetongue. Peak levels were found some eight days after the peak of the febrile reaction and elevated levels persisted for some eight days thereafter.

Further studies on this subject are now reported. In addition to the enzymes listed above, plasma levels of creatine phosphokinase (CPK) and arginase were also determined.

METHODS

Plasma Samples

Sheep passing through artificially induced bluetongue reactions were made available for bleeding by the virology section of this Institute. Various strains were used and the reactions varied from fatal to extremely mild. Samples were taken at intervals from the date of injection, or the beginning of the febrile reaction, to up to 14 days after the fever had passed off. Only figures from animals which survived the febrile period by at least two days were used. The incubation period, febrile period and post-febrile periods were determined for each sheep from its temperature chart. Figures for the fatal and non-fatal cases are presented separately. In all, figures from 24 reactors, 6 fatal and 18 non-fatal, are presented.

Enzyme Assays

The methods used were as given in the following references:— Ald[2,3], LDH[4], CPK[5], GOT[6], GPT[6] and arginase[7]. The units used in the text are as defined in the respective publications with the exception of LDH where the unit is as defined by Wagner and Brown[8].

RESULTS

TABLE 1.—PLASMA ENZYME LEVELS DURING BLUETONGUE REACTIONS

	Aldolase		LDH		CPK		GOT		GPT	
	Mean (n)	Range	Mean (n)	Range	Mean (n)	Range	Mean (n)	Range	Mean (n)	Range
Fatal Cases (6)										
Period										
Incubation.....	— (1)	16	—	—	— (1)	17	— (1)	83	— (1)	38
Febrile period.....	15 (6)	10– 21	600 (8)	330– 900	15 (8)	7–27	136 (8)	107–215	31 (8)	9– 59
1— 3 days post-F.R...	— (2)	53–582	— (2)	750–6080	— (2)	43–548	— (2)	220–557	— (2)	61–337
4—6 ,, ,, ,,....	195 (4)	35–362	2390 (4)	750–3570	134 (4)	24–318	387 (4)	280–425	177 (4)	67–225
7—10 ,, ,, ,,....	— (2)	325–600	— (2)	4010–5000	— (2)	253–358	— (2)	575–650	— (2)	330–355
Recovered Cases (18)										
Period										
Incubation.....	19 (10)	10– 30	436 (11)	90– 920	19 (11)	0– 50	120 (7)	96–146	20 (14)	6– 32
Febrile period.....	19 (18)	7–48	584 (18)	230–1100	19 (15)	6– 42	135 (18)	83–168	47 (18)	8–150
1— 3 days post-F.R....	104 (7)	35–155	1063 (7)	540–2700	93 (4)	15–194	275 (7)	200–345	108 (8)	53–187
4— 6 ,, ,, ,,...	195 (4)	8–274	884 (17)	510–1740	30 (16)	0–169	250 (18)	125–470	74 (16)	21–128
7—10 ,, ,, ,,...	81 (15)	13–550	906 (16)	330–2720	30 (13)	0–123	235 (15)	160 337	88 (17)	34–220
11—14 ,, ,, ,,...	14 (16)	5–34*	651 (16)	335–1200	12 (14)	0–30	160 (16)	106–302	40 (18)	5– 63

Figures in brackets indicate number of animals from which readings were obtained.
"post-F.R." = days after the febrile period
*Does not include readings of 105 and 495 from 2 sheep
LDH—Lactic dehydrogenase.
CPK—Creatine phosphokinase
GOT—Glutamic oxalo-acetic transaminase
GPT—Glutamic pyruvic transaminase.

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Aldolase

Wagner and Brown[8] found the upper limit for apparently normal sheep at Onderstepoort to be 37. All readings taken during the incubation period fell below this level and only one (48) taken in the febrile period was above it. During the post-febrile period, one of the six fatal cases showed only a moderate rise to 53 while the peaks for the others ranged from 240 to 600.

During this period 16 of the 18 recovered cases showed elevations, the peaks ranging from 63 to 550. In two cases the rise persisted into the 10 to 14 day post-febrile period.

Lactic Dehydrogenase

The upper limit of the normal range is taken as 750[8]. During the incubation period two sheep showed higher figures (830 and 920). During the febrile period, three of the fatal cases showed high levels (790, 820 and 900) while three recovered cases showed 950, 1030 and 1100 respectively. During the post-febrile period one fatal case showed a peak figure of only 750 (from 460 previously) while the peaks for the others were from 2640 to 6080. Of the 18 recovered cases, four failed to show any significant rise. The highest figure obtained from this group was 2720. Four animals still showed high figures after the 11th post-febrile day.

Creatine Phosphokinase

As no figures for normal sheep at Onderstepoort are available, the figures obtained during the incubation period will be used as criteria. The mean was 19 (0-50). None of the sheep showed a rise during the febrile period. All the fatal cases showed very high figures during the post-febrile period (92 to 358) whereas only eight of the 18 recovered cases showed a rise (70 to 194).

Glutamic Oxalacetic Transaminase

The upper normal limit is taken as 180[8]. Of the fatal cases, one showed a figure of 215 during the febrile period and all six showed post-febrile reactions (220 to 650). None of the recovered cases showed elevated levels during the incubation or febrile periods but 15 of the 16 showed post-febrile increases (200 to 470).

Glutamic Pyruvic Transaminase

The upper limit is taken as 60[8]. No figures above this were obtained during the incubation period. During the febrile period six sheep, all of which recovered, showed figures above 60 (66-150). One of the fatal cases showed a maximal figure of only 61 during the post-febrile period but peak figures from the other five varied from 195 to 355. During this period 17 of the 18 recovered cases showed elevated levels (65-150).

Arginase

The plasma arginase level was determined on 32 samples showing high enzyme levels. No increase in arginase was found in any of them.

Discussion

Consistency of Plasma Enzyme Elevation

All six fatal cases which survived the febrile reaction, showed significant rises in Ald., LDH, CPK, GOT and GPT.

The consistency of elevations in the various enzymes examined in the recovered cases is shown in Table 2.

Table 2.—Consistency of significant plasma enzyme elevations in Bluetongue Recovered Cases.

No. of Sheep.	Ald	LDH	CPK	GOT	GPT
6	+	+	+	+	+
6	+	+	—	+	+
3	+	—	—	+	+
1	—	+	+	—	+
1	+	—	+	—	—
1	—	+	—	—	+
Tot. 18	16	14	8	15	17
+ =	>37	>750	>50	>180	>60

Tot. = Total number of sheep showing significant elevations.

As already stated, the recovered cases covered a wide range of severity of reaction from fever on one day only to typical blue-tongue. A wide range of plasma enzyme elevations would therefore be expected. Despite this, all sheep showed elevations in at least two of the enzymes. The most consistent rises were in GPT and Aldolase. CPK showed up in only 8 out of 16 cases.

Origin of the Plasma Enzymes

The main organs which could be involved in the elevations of plasma enzyme levels described are skeletal muscle, liver and myocard. The fact that CPK reached very high levels in many cases indicates a myopathy. As pointed out in the preliminary communication, no significant or consistent electrocardiographic abnormalities could be detected in post-febrile bluetongue cases which would appear to rule out the myocard as a major source of the enzymes. The absence of any rise in arginase would appear to eliminate acute liver pathology as do the absence of hyperbilirubinaemia and normal bromsulphthalein excretion as previously reported[1]. The cause of the rise in these plasma enzymes would therefore appear to be the skeletal myopathy which forms a prominent feacture of the bluetongue syndrome.

Determination of plasma enzymes may be of value in the presumptive diagnosis of bluetongue in the post-febrile and post-viraemic periods.

The possibility of correlating plasma enzyme levels with the virulence of strains intended for use in vaccine production will be investigated when suitable material is available.

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A REPORT ON SOME LONG TERM EFFECTS OF THYRO-PARATHYROIDECTOMY IN A MERINO WETHER.

P. C. BELONJE*.

SUMMARY

Thyro-parathyroidectomy was performed on a four-year old merino wether. Radioactive iodine uptake ten months later confirmed a hypothyroid state. The animal became progressively more lethargic but no symptoms of myxoedema were noticed, nor was there a pronounced increase in plasma cholesterol.

Nine days before death, i.e. fourteen months after the operation, analyses revealed an increase in plasma globulin and in red cell sedimentation rate. The plasma calcium, inorganic phosphate, magnesium, transaminases and blood volume and haemoglobin were all normal.

Inexplicably, the plasma calcium rose transiently about eleven days after the operation.

INTRODUCTION

On 22.10.1965 thyro-parathyroidectomy was performed on a four year old merino wether. The recovery was uneventful and the sheep was kept in a stall and fed teff hay *ad lib* (Ca 0.28%; P 0.17%; Mg 0.16%) and occasionally small quantities of a balanced meal ration (Ca 0.68%; P 0.44%; Mg 0.08%).

The animal remained clinically normal for about eleven months, after which it became progressively more lethargic until it died on 18.12.1966. During the fourteen months it's weight had increased from 109 to 119 lbs.

A post mortem examination revealed a slight tumor splenis, seven encapsulated air cysts in the lungs, but no signs of myxoedema. No remaining thyroid or parathyroid tissue could be found by macroscopic or microscopic examination.

RESULTS AND DISCUSSION

1. *Radioactive iodine uptake.* Commencing on 23.8.1966 a 72-hour thyroid I^{131} uptake was performed on this sheep and on a normal merino wether as control. The results are presented in the following table and demonstrate that although there was some concentration of iodine in the neck, the experimental animal was indeed in a hypothyroid condition.

TABLE.—THE PERCENTAGE RADIOACTIVE IODINE UPTAKE (USING THE INNER THIGH AS BLANK)

Hour	Experimental Sheep	Normal Sheep
2.....	0.5	2.9
28.....	6.8	17.5
52.....	10.3	28.7
72.....	12.4	28.3

2. *Plasma cholesterol.* This rose from 62.8 mg % at the time of the operation to 81.0 mg % five months later and was found to be 90.0 mg % nine days before death. Whether this increase is significant in the sheep, is unknown. In man plasma cholesterol concentrations of up to 400-700 mg % are found after total extirpation of the thyroid glands[1].

3. *Total plasma proteins.* These rose gradually from 8.10g % (22.10.65) to 8.70g % (14.3.1966) and finally to 9.14g % (9.12.1966). Plasma electrophoresis conducted on a sample taken on 9.12.1966 revealed the following:

   Albumin 2.29 g %

   Globulins 6.85 g % = α_1 0.49 g %
   α_2 0.98 g %
   β 3.42 g %
   γ 1.96 g %

   As the analysis was performed on plasma, the fibrinogen was probably incorporated in the β-globulin fraction. Nevertheless it is considered that this fraction was abnormally high[2].

4. *Red cell sedimentation rate.* On 9.12.1966 this was 38 m.m. per hour, a significant increase over the normal mean of 2 m.m. per hour.

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This rise is probably the result of the increased plasma globulin fraction and a lowered packed cell volume of 27%[3].

5. *Plasma calcium.* Prior to the operation the plasma calcium concentration was 10.0 mg % and remained virtually unchanged for the first five postoperative days. This was followed by an inexplicable transient increase reaching a peak eleven days after the operation (13.1 mg %) after which it gradually returned to preoperative levels. Nine days before death the concentration was 9.5 mg %.
6. *Plasma inorganic phosphate and magnesium.* No particular trends were noticed. The former varied between 4.1-6.0 mg % and the latter between 1.75-2.25 mg % throughout the period.
7. *Other analyses.* These were performed on samples drawn nine days before death and included blood urea nitrogen (24 mg %), the serum transaminases (SGOT 132 and SGPT 18 King Units), haemoglobin (8.25 g %) and blood volume (1557 ml.) all of which fell within the range of normality.

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THE EFFECT OF DIETHYLSTILBOESTROL ON THE WEIGHT GAIN AND FEED CONVERSION OF CATTLE WHEN INCLUDED IN HIGH ENERGY DIETS CONTAINING NATURAL PROTEIN OR NON-PROTEIN NITROGEN AS THE PROTEIN SOURCE.

D. K. SHONE and R. L. CAIN*.

SUMMARY

The feeding of diethylstilboestrol to 11 month old fattening steers on a high energy natural protein diet resulted in a 10 per cent increase in liveweight gain and a 10 per cent improvement in feed conversion. Diethylstilboestrol fed to similar steers on a high energy non-protein nitrogen diet resulted in a 5.38 per cent increase in liveweight gain and a 3.8 per cent improvement in feed conversion.

The only roughage fed was that present in the maize, cob and sheath meal milled through a ½ inch screen. No problem was experienced with feed intake or bloating.

The best economic return was obtained by feeding diethylstilboestrol with the natural protein diet. This also provided the best feed conversion of 7.1 and an average daily weight gain of 2.98 lb.

INTRODUCTION

The use in the United States of America of diethylstilboestrol in the feed and in Great Britain of hexoestrol implants is well recognized in bringing about an increase of between 10 and 15 per cent in weight gains and a similar improvement in feed conversion in fattening cattle. It has been estimated that 90 per cent of fattening cattle in these two countries are given oestrogenic growth stimulants.

In South Africa the use of oestrogens for fattening slaughter animals has been banned for many years. This ban has some merit when applied to the implants and particularly as used in poultry, because the incorrect siting of the implants in poultry may result in large residues being found in the carcass and also because much of the offals are eaten by a large section of our population. There does not appear to be any justification for this ban to include the oral administration of diethylstilboestrol where complete withdrawal is possible, and no traces of the compound can be found in cattle carcasses after 12 hours[1, 2]. It is rare for cattle to be slaughtered within 24 hours of leaving a farm and thus a further safeguard is provided against even the minimal traces found when diethylstilboestrol is fed with no withdrawal period.

This work was undertaken in order to establish that feed conversion and liveweight gains comparable to those reported from overseas could be achieved under South African conditions by feeding diethylstilboestrol.

The two diets are probably the simplest that can be devised and were based upon those used by Shone and Buchanan[3]. This type of diet gave weight gains and feed conversions comparable to those reported from overseas and makes maximum use of maize (cob and sheath meal), the major South African grain crop.

MATERIAL AND METHODS

Cattle

Forty ¾ Sussex and ¼ Afrikander steers approximately 11 months of age were used in the trial. The steers were selected from a herd of several hundred to ensure maximum uniformity. On arrival the steers were vaccinated against black quarter and grazed on *Eragrostis teff* pastures for approximately two months. The cattle were treated with 15 mg of tetramisole per kg bodyweight when placed in the feeding pens.

Diethylstilboestrol

The diethylstilboestrol used was in the form of a premix** containing 10 mg of the compound per ounce. The product had been subjected to the usual stability tests at room and at elevated temperatures and humidity.

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Feeds

All the rations had isonitrogenous values equivalent to 14 per cent crude protein.

The maize, cob and sheath meal was milled in a hammermill with a ½ inch screen. No other form of roughage was fed. The urea was in the form of prills, as is supplied for fertilizer and feed purposes. As the vitamin A status of the steers was not known, vitamin A was added to all rations at the rate of 6 million units per ton of feed. The composition of the feeds is presented in Table I. The mineral premix is identical to the one used by Shone and Buchanan[3].

Individual liveweights were recorded weekly and the initial and terminal liveweights were the mean of 3 weights taken at 24 hour intervals.

No diethylstilboestrol was fed for the 48 hours prior to sending the cattle to the abattoir.

Results

The weekly mean liveweights and mean daily quantities of feed and water consumed are presented in Table II. The feed conversion, liveweight gain, dressed carcass weights and dressing percentages are also given in this table.

Table I.—Compositon of natural protein and non-protein nitrogen feeds

	Natural Protein feed		Non-protein nitrogen feed	
	Pen 1A.	Pen 1B.	Pen 2A.	Pen 2 B.
Maize, cob and sheath meal	1,570	1,564	1,883	1,887
Ground nut oil cake meal	360	360	0	0
Urea	0	0	47	47
Mineral premix	70	70	70	70
Vitamin premix	5	5	5	5
Diethylstilboestrol premix	0	6¼	0	6¼

Design

The 40 Sussex steers were randomly assigned on a weight basis to each of 4 pens. Two pens of 10 head each were fed on the natural protein diet with and without diethylstilboestrol and two pens of 10 head each were fed on a non-protein nitrogen diet with and without diethylstilboestrol.

The feeding period covered 122 days and during the first week the feed was restricted, but thereafter it was available on an *ad lib* basis. The quantity of feed added to the trough was recorded daily and the quantity remaining at the end of the week weighed back. Similar recordings were made for the drinking water.

An examination of these figures shows that of the cattle fed on the natural protein group, those which also received the diethylstilboestrol had a 10.37 per cent advantage in liveweight gained and a 10.13 per cent improvement in feed conversion when compared to the steers which received no diethylstilboestrol. The differences recorded for the non-protein nitrogen diet were not as great. The steers which received the diethylstilboestrol had a 5.38 per cent liveweight gain advantage and a 3.8 per cent improvement in feed conversion over the steers which received no diethylstilboestrol.

An efficacy factor obtained by dividing the liveweight gain by the feed conversion calculated

Table II.—Details of mean liveweight gains, feed and water consumption, feed conversion, dressed weights and dressing percentage of cattle fed for 122 days on natural protein and non-protein nitrogen diets with and without diethylstilboestrol

	Natural Protein feed		Non-protein nitrogen feed	
	No stilboestrol	with stilboestrol	No stilboestrol	with stilboestrol
Initial liveweight lb	492.0	484.6	477.2	499.0
Final liveweight lb	821.4	848.1	795.0	833.7
Total liveweight gain lb	329.2	363.5	317.8	334.7
Average daily gain lb	2.7	2.97	2.6	2.74
Feed consumed lb	2,598	2,582	2,581	2,613
Feed conversion	7.9	7.1	8.1	7.8
Water consumed gallons	708.7	744.6	717.6	756.6
Dressed weight lb	456.1	467.3	430.1	458.2
Dressing percentage	55.5	55.1	54.1	55.0

for each group, also shows the influence of diethylstilboestrol on liveweight gains and feed conversion.

		Efficiency Factor
Pen 1 A.	Natural Protein feed	41.7
Pen 1 B.	Natural Protein feed plus diethylstilboestrol	51.2
Pen 2 A.	Non-Protein nitrogen feed	39.1
Pen 2 B.	Non-Protein nitrogen feed plus diethylstilboestrol	42.9

The advantage of the natural protein feed plus diethylstilboestrol group over the other group is 22.78 per cent, while that of the non-protein nitrogen feed plus diethylstilboestrol group over the other non-protein nitrogen feed group is 9.71 per. cent.

All the cattle except one were graded super at slaughter. One was graded prime A because of poor conformation. The average price received for the super carcasses was R22.91 per 100 lb dressed weight.

Details of the costs involved in this trial are presented in Table III and a simple revenue and expenditure account in Table IV. It will be noted that no costs have been allocated for interest on capital, depreciation, labour etc., and the profits given cannot be regarded as true profits.

Discussion

The advantage of including diethylstilboestrol in the feed of cattle, whether on a natural protein or urea diet, has been clearly demonstrated. The advantage to be derived from the use of diethylstilboestrol was greater with the natural protein diet in improved feed conversion and average liveweight gain. In this trial the differences in actual profits made, ranged from R3.64 to R6.19 per head (69.8 per cent increase) for the natural protein diet and from R4.62 to R6.92 (49.7 per cent increase) for the urea diet.

If the feed conversion and liveweight gain figures are considered, it is obvious that the above sums are not a true reflection of the differences in profits between the natural protein and urea diets, and this is confirmed by setting up a hypothetical case based upon the liveweight gain, dressing percentage, feed conversion, feed cost and return per 100 lb dressed carcass weight obtained in this trial. If steers on a natural protein diet without diethylstilboestrol showed a liveweight gain of 350 lb. over 122 days, then a similar steer fed on diethylstilboestrol would return an extra net profit of R9.07 per head. Similar steers on non-protein nitrogen diet would, when fed on diethylstilboestrol show an extra net profit of R3.23 per head over those not receiving die-

Table III.—Cost of material used in the trial

Maize, cob and sheath meal	1.85 cents per lb
Urea	3.35 cents per lb
Ground nut cake meal	3.11 cents per lb
Mineral premix cost per ton feed	R 3.95
Vitamin A premix cost per ton of feed	R 0.34
Natural Protein feed with diethylstilboestrol	2.275 cents per lb
Natural Protein feed without diethylstilboestrol	2.227 cents per lb
Non-Protein nitrogen feed with diethylstilboestrol	2.083 cents per lb
Non-Protein nitrogen feed without diethylstilboestrol	2.035 cents per lb
Steers, cost per lb liveweight	11.0 cents per lb

Table IV.—The direct expenditure and revenue return obtained in the trial per 10 steers

	Natural Protein Feed		Non-Protein Nitrogen Feed	
	Pen 1 A.	Pen 1 B.	Pen 2 A.	Pen 2 B.
	Without diethylstilboestrol	10 mgm. diethylstilboestrol per day	Without diethylstilboestrol	10 mgm. diethylstilboestrol per day
Expenditure				
Steers	R 541.42	R 533.06	R 524.92	R 548.90
Feed	R 578.46	R 587.05	R 525.23	R 544.08
Total	R 1,119.88	R 1,120.11	R 1,050.15	R 1,092.98
Revenue	R 1,156.32	R 1,181.99	R 1,092.48	R 1,161.14
Revenue less expenditure	R 36.44	R 61.88	R 42.23	R 69.16

Price received for super carcasses was R22.91 per 100 lbs.
Price received for one prime A carcasses was R21.80 per 100 lbs.
Additional sums were received for offal, hides etc.

thylstilboestrol. The cost of the diethylstilboestrol at 1 cent per day was included in the cost of the feed.

The differences between these theoretical calculations and the actual figures are due to differences in starting liveweights between groups.

Shone and Buchanan[3] demonstrated that a non-protein diet provides a greater economic return than does a natural protein diet, despite a lower production, because of the lower feed costs. This was confirmed in this trial where the profits from the non-protein nitrogen diet group was 16 per cent greater than that for the natural protein nitrogen diet group when no diethylstilboestrol was fed. The feeding of diethylstilboestrol with a natural protein diet reversed the effect and resulted in greater profits and more meat from less feed.

If the use of diethylstilboestrol is approved, it will be of major benefit to the cattle fattening industry and the country as a whole.

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BOOK REVIEW

THE IXODID TICKS OF TANZANIA by G. H. YEOMAN and JANE B. WALKER. Published by Commonwealth Institute of Entomology, London 1967, pp 215, Maps 46. Publ. Price 45/-.

At the African Regional Conference held in Johannesburg in 1949 it was recommended that a tick survey of the faunal zones South of the Sahara be undertaken. It was stressed that we need to know much more of the biology of the ticks and their responses to seasonal macro- and micro-climatic conditions, and that a correlation of the information gathered in all aethiopian territories was essential for the tick and tick-borne diseases problem to be tackled in a concerted manner.

This is the first work, other than in South Africa, planned and carried out to fill in the gaps. The local tick species are mapped and analysed against a comprehensive background of physiography, vegetation zones, annual rainfall, duration of dry periods, agricultural and husbandry practices, and to a certain extent availability of hosts. Infestation rates, predilection sites and disease relationships are discussed.

The book achieves more than it sets out to do, that is to serve as a handbook for Tanzanian Veterinarians and local authorities on which to base recommendations for regional husbandry and dipping practices. The information gathered allows neighbouring countries to apply Tanzanian findings wherever somewhat similar conditions prevail. Our knowledge of African ticks has been taken a long step forward.

G.T. June, 1967.

Editor's note.

The dedication to the above book reads as follows:-

This work is dedicated with
respect and affection to
GERTRUD THEILER
pioneer of studies on
Zoogeography of
African ticks

This is indeed an elegant and fitting tribute to an Honorary Associate Member of our association.

MODIFICATIONS TO THE LARVAL ANTHELMINTIC TEST

R. K. REINECKE† and *P. J. S. ANDERSON‡

SUMMARY

Two modifications to the larval anthelmintic test are described:

1. Susceptible worm-free sheep were infested orally every day with infective larvae and divided into groups. In one group worms were in the third stage or third moult when treated; in the other groups fourth stage, fourth moult and early fifth stage worms were present.

2. The controls were killed either on the day the other sheep were treated (Day 0) or within two days of treatment (Day + 1 or Day +2). The treated sheep were killed either two or three days after treatment. For worms in the third stage or third moult on Day 0 this method was unsatisfactory: fewer worms were recovered and most of them were in an earlier stage of development on Day 0 than on Day + 2. On the other hand, worm burdens in Day 0 controls infested with fourth stage larvae or more advanced stages were directly comparable with those of treated sheep killed on Day + 2 or Day + 3.

INTRODUCTION

Recently a new compound, *trans*-1-methyl-2 [2-(a-thienylvinyl)] 1, 4, 5, 6-tetrahydropyrimidine tartrate, common name pyrantel tartrate*, has been synthesized[1].

It has been reported that this compound is effective against the immature stages of parasitic nematodes[2].

Anthelmintic tests were therefore carried out with this new compound using two modifications of the larval anthelmintic test described by Reinecke[3]. Instead of infesting sheep regularly so that worms were in all stages of development at treatment the first modification was introduced. The anthelmintic was tested against worms in the third stage and third moult in one group of sheep as distinct from worms in the fourth stage, fourth moult and early fifth stage in other groups. Secondly, controls were killed either on the same day or within two days of treatment. Treated sheep were killed two or three days after treatment. In the past most of the animals were killed at least six days after treatment.

EXPERIMENT 1

Materials and methods

1. Twenty-four weaned Dorper (Dorset Horn x Black Head Persian) and Merino sheep born, raised and maintained worm-free were used. They were divided into three groups and dosed *per os* with infective larvae of *Oesophagostomum columbianum, Haemonchus contortus* and *Trichostrongylus colubriformis* as follows:

Group (a) Eight sheep each dosed from Day — 3 to Day — 1.

Group (b) Eight sheep each dosed from Day — 12 to Day — 4.

Group (c) Eight sheep each dosed on seven separate occasions from Day — 26 to Day — 12 with infective larvae of *O. columbianum* only.

2. On Day 0 half of each group was treated intraruminally with pyrantel tartrate at 25 mg/kg.

3. Details of the experimental design, including the slaughter of the various sheep, are summarized in Table 1.

4. The post mortem procedures have been described[4]. The ingesta of the fore-stomachs was also examined for worms in Sheep 1.

Results (See Table 2)

Controls: Group (a) One to three-day old worms

Fewer worms were recovered from the Day 0 control (Sheep 1) than the Day + 2 controls (Sheep 2, 3 and 4). With the exception of two worms in the third moult in Sheep 2 all the *O. columbianum* were still in the third stage in the Day + 2 controls. On Day 0 most of the *T. colubriformis* were in the third stage. By Day + 2, however, the majority had developed to the third moult or early fourth stage. With the exception of four third stage larvae in one Day + 2 control

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TABLE 1.—EXPERIMENT 1: EXPERIMENTAL DESIGN.

Group	Day	No. of infective larvae dosed to each sheep.		
		O. columbianum	*H. contortus*	*T. colubriformis*
A	—3	200	1,000	1,000
	—2	,,	,,	,,
	—1	,,	,,	,,
	Total	600	3,000	3,000
	0	Killed Sheep 1: Day 0 Control Treated Sheep 5, 6, 7 & 8 with pyrantel tartrate at 25 mg/kg intraruminally		
	+2	Killed Sheep 2, 3 & 4: Day +2 Controls		
	+3	Killed Sheep 5, 6, 7 & 8 Treated on Day 0		
B	—12	100	250	250
	—11	,,	,,	,,
	—10	,,	,,	,,
	— 9	,,	,,	,,
	— 8	,,	,,	,,
	— 7	,,	,,	,,
	— 6	,,	,,	,,
	— 5	,,	,,	,,
	— 4	,,	,,	,,
	Total	900	2,250	2,250
	0	Killed Day 0 Controls: Sheep 9, 10, 11 & 12 Treated Sheep 13, 14, 15 & 16 with pyrantel tartrate at 25 mg/kg intraruminally		
	+ 3	Killed Sheep 13, 14, 15 & 16 treated on Day 0		
C	—26	100	—	—
	—24	,,	—	—
	—21	,,	—	—
	—19	,,	—	—
	—17	,,	—	—
	—14	,,	—	—
	—12	,,	—	—
	Total	700	—	—
	0	Killed Day 0 Controls: Sheep 17 & 18. Treated Sheep 21, 22, 23, & 24 with pyrantel tartrate at 25 mg/kg intraruminally		
	+ 1	Killed Day +1 Controls : Sheep 19 & 20		
	+ 3	Killed Sheep 21, 22, 23 & 24 treated on Day 0		

(Sheep 2) all the *H. contortus* were in the early fourth stage by Day + 2 (Table 2).

Group (b) Four to twelve-day old worms

The controls were killed on Day 0. Most of the *O. columbianum* were in the fourth stage, some in the third stage and a few in the third moult. Only one sheep (No. 9) contained 12 worms in the fourth moult. The great majority of *H. contortus* and *T. colubriformis* were in the fourth and fifth stage.

Group (c) *O. columbianum* 12-26 days old

The controls were killed on Day 0 and Day + 1 and in both groups the worms were at similar stages of development, i.e. the fourth stage. *Treated sheep*: The anthelmintic was 79.3 - 100.0 per cent effective against *H. contortus* and 98.1 - 100.0 per cent effective against all stages of *T. colubriformis*. It was, however, only 14.0 per cent effective against the early fourth stage of *O. columbianum* though it rose again to 93.5 per cent against the fourth moult of this species.

TABLE 2.—EXPERIMENT 1: WORMS RECOVERED POST MORTEM

GROUP	Sheep No.	*O. columbianum* Stage of development					*H. contortus* Stage of development					*T. colubriformis* Stage of development				
		*L3	3 M	L 4	4 M	5	L 3	3 M	L 4	4 M	5	L 3	3 M	L 4	4 M	5
Group (*a*) One to three-day old worms at treatment																
CONTROLS..... Day 0	1	212	0	0	—	—	20	11	37	—	—	396	25	0	—	—
Day +2	2	347	2	0	—	—	4	0	708	—	—	37	435	141	—	—
Day +2	3	293	0	0	—	—	0	0	358	—	—	58	449	218	—	—
Day +2	4	453	0	0	—	—	0	0	555	—	—	9	288	187	—	—
TREATED..... Day +3	5	141	20	11	—	—	0	0	22	—	—	0	0	0	—	—
Day +3	6	123	11	11	—	—	0	0	0	—	—	0	0	0	—	—
Day +3	7	228	19	27	—	—	0	0	65	—	—	0	0	0	—	—
Day +3	8	143	40	23	—	—	0	0	143	—	—	0	0	2	—	—
Average Reduction.....		51.3%	0.0%	0.0%	—	—	100.0%	100.0%	86.1%	—	—	100.0%	100.0%	99.6%	—	—
Group (*b*) Four to twelve-day old worms at treatment																
CONTROLS..... Day 0	9	99	28	196	12	0	—	—	468	41	156	—	17	394	177	266
Day 0	10	129	3	165	0	0	—	—	396	116	132	—	1	696	113	202
Day 0	11	93	13	33	0	0	—	—	573	66	99	—	0	477	27	233
Day 0	12	138	45	164	0	0	—	—	675	134	242	—	12	276	12	176
TREATED..... Day +3	13	66	9	105	6	0	—	—	0	0	0	—	0	0	0	0
Day +3	14	40	4	111	0	4	—	—	3	0	3	—	0	0	0	3
Day +3	15	77	7	102	4	0	—	—	42	19	107	—	0	6	1	8
Day +3	16	124	7	162	0	0	—	—	12	3	20	—	0	17	3	6
Average Reduction.....		33.1%	69.5%	14.0%	16.7%	0.0%	—	—	97.3%	93.8%	79.3%	—	100.0%	98.7%	98.8%	98.1%
Group (*c*) Twelve to twenty-six-day old worms at treatment																
CONTROLS..... Day 0	17	6	15	131	13	30	—	—	—	—	—	—	—	—	—	—
Day 0	18	0	5	185	12	72	—	—	—	—	—	—	—	—	—	—
Day +1	19	20	3	126	15	204	—	—	—	—	—	—	—	—	—	—
Day +1	20	0	0	158	9	71	—	—	—	—	—	—	—	—	—	—
TREATED..... Day +3	21	36	0	149	3	61	—	—	—	—	—	—	—	—	—	—
Day +3	22	10	5	60	0	11	—	—	—	—	—	—	—	—	—	—
Day +3	23	8	0	13	0	0	—	—	—	—	—	—	—	—	—	—
Day +3	24	3	0	56	0	0	—	—	—	—	—	—	—	—	—	—
Average Reduction.....		0.0%	78.3%	53.7%	93.5%	80.9%	—	—	—	—	—	—	—	—	—	—

* L 3 and L 4 = Third and fourth stage larvae.
M = Moult
5 = Fifth stage

TABLE 3.—EXPERIMENT 2: EXPERIMENTAL DESIGN

Group	Day	No. of infective larvae dosed to each sheep		
		C. ovina	*O. circumcincta*	*N. spathiger*
A	— 3	200	1,000	—
	— 2	,,	,,	—
	— 1	,,	,,	—
	Total	600	3,000	—
	0	Killed Sheep 25: Day 0 Control Treated Sheep 29, 30, 31 & 32 with pyrantel tartrate at 25 mg/kg intraruminally		
	+ 2	Killed Sheep 26, 27 & 28: Day +2 Controls.		
	+ 3	Killed Sheep 29, 30, 31 & 32 treated on Day 0		
B	—12	100	250	—
	—11	,,	,,	—
	—10	,,	,,	—
	— 9	,,	,,	—
	— 8	,,	,,	—
	— 7	,,	,,	—
	— 6	,,	,,	—
	— 5	,,	,,	—
	— 4	,,	,,	—
	— 3	—	—	500
	— 2	—	—	,,
	— 1	—	—	,,
	Total	900	2,250	1,500
	0	Killed Sheep 33: Day 0 Control Treated Sheep 37, 38, 39 & 40 with pyrantel tartrate at 25 mg/kg intraruminally		
	+ 2	Killed Sheep 34, 35 & 36 Day +2 Controls Killed Sheep 37 & 38 treated on Day O		
	+ 3	Killed Sheep 39 & 40 treated on Day 0		
C	—26	100	—	—
	—24	,,	—	—
	—21	,,	—	—
	—19	,,	—	—
	—17	,,	—	—
	—14	,,	—	—
	—12	,,	—	150
	—11	—	—	,,
	—10	—	—	,,
	— 9	—	—	,,
	— 8	—	—	,,
	— 7	—	—	200
	— 6	—	—	,,
	— 5	—	—	400
	— 4	—	—	,,
	Total	700	—	1,950
	0	Killed Sheep 41 & 42 Day 0 Controls Treated Sheep 45, 46, 47 & 48 with pyrantel tartrate at 25 mg/kg intraruminally		
	+ 1	Killed Sheep 43 & 44 Day +1 Controls		
	+ 2	Killed Sheep 45, 46, 47 & 48 treated on Day 0.		

TABLE 4.—EXPERIMENT 2: WORMS RECOVERED POST MORTEM

GROUP	Sheep No.	*C. ovina* Stage of development					*O. circumcincta* Stage of development					*N. spathiger* Stage of development					
		*L 3	3 M	L 4	4 M	5	L 3	3 M	L 4	4 M	5	L 3	3 M	L 4	4 M	5	A
Group (a) One to three-day old worms at treatment																	
CONTROLS..... Day 0	25	83	0	—	—	—	430	42	0	—	—	—	—	—	—	—	—
Day +2	26	0	0	—	—	—	127	41	72	—	—	—	—	—	—	—	—
Day +2	27	0	1	—	—	—	47	17	24	—	—	—	—	—	—	—	—
Day +2	28	0	0	—	—	—	99	143	99	—	—	—	—	—	—	—	—
TREATED..... Day +3	29	0	0	—	—	—	0	0	0	—	—	—	—	—	—	—	—
Day +3	30	0	0	—	—	—	0	0	0	—	—	—	—	—	—	—	—
Day +3	31	0	0	—	—	—	0	0	0	—	—	—	—	—	—	—	—
Day +3	32	0	0	—	—	—	0	0	0	—	—	—	—	—	—	—	—
Average Reduction.....		100.0%	100.0%	—	—	—	100.0%	100.0%	100.0%	—	—	—	—	—	—	—	—
Group (*b*) Four to twelve-day old worms at treatment												One to three-day old worms at treatment					
CONTROLS..... Day 0	33	26	—	20	—	—	—	—	19	3	2	71	0	5	—	—	—
Day 2	34	0	—	306	—	—	—	—	59	3	18	72	17	57	—	—	—
Day +2	35	6	—	204	—	—	—	—	17	1	2	100	148	37	—	—	—
Day +2	36	20	—	266	—	—	—	—	4	4	4	81	0	0	—	—	—
TREATED..... Day +2	37	0	—	0	—	—	—	—	0	0	0	0	0	0	—	—	—
Day +2	38	0	—	0	—	—	—	—	3	0	0	0	0	0	—	—	—
Day +3	39	0	—	16	—	—	—	—	8	0	5	3	3	6	—	—	—
Day +3	40	0	—	0	—	—	—	—	3	0	3	0	0	0	—	—	—
Average Reduction.....		100.0%	—	98.0%	—	—	—	—	86.0%	100.0%	69.2%	99.7%	98.1%	93.9%	—	—	—
Group (*c*) Twelve to twenty-six-day old worms at treatment												Four to twelve-day old worms at treatment					
CONTROLS..... Day 0	41	—	—	145	4	38	—	—	—	—	—	0	—	22	3	14	0
Day 0	42	—	—	205	10	38	—	—	—	—	—	0	—	723	58	29	15
Day +1	43	—	—	226	12	27	—	—	—	—	—	0	—	572	48	121	15
Day +1	44	—	—	160	0	30	—	—	—	—	—	3	—	658	0	0	0
Day 0	45	—	—	0	5	7	—	—	—	—	—	0	—	0	3	0	0
Day +2	46	—	—	0	5	0	—	—	—	—	—	0	—	0	0	0	0
Day +2	47	—	—	0	0	1	—	—	—	—	—	0	—	0	3	0	0
Day +2	48	—	—	1	0	0	—	—	—	—	—	0	—	0	0	0	0
Average Reduction.....		—	—	99.9%	61.5%	94.0%	—	—	—	—	—	100.0%	—	100.0%	94.5%	100.0%	100.0%

* L 3 and L 4 = Third and fourth stage larvae
M = Moult
5 = Fifth stage.

Experiment 2

Materials and Methods

1. Twenty-four weaned Dorper sheep born, reared and maintained worm-free were used. They were divided into three equal groups and infested orally with infective larvae of *Chabertia ovina* and *Ostertagia circumcincta*. Details of the infestations are summarized in Table 3; with one exception the design resembles Experiment 1. Initially infective larvae of *Nematodirus spathiger* were not available but they were harvested in time to be included in Group (b) and were dosed daily to this Group from Day — 3 to Day — 1 and to Group (c) from Day — 12 to Day — 4 (Table 3).

2. Pyrantel tartrate was dosed by intraruminal puncture at 25 mg/kg to half the sheep in each group on Day 0. Details of their infestation, treatment and slaughter are included in the experimental design (Table 3).

3. The ingesta of the fore-stomachs was included in the specimens examined in Sheep 25 and 33.

Results (See Table 4)

Controls: Group (a) One to three-day old worms

In two sheep moderate numbers of *C. ovina* (1-83) were found but in others they were entirely absent, which strongly suggested that the larvae were not viable. The number of third stage larvae of *O. circumcincta* recovered from the Day 0 control (Sheep 25) was greater than the total number of worms recovered at Day + 2 in any of the other controls (Sheep 26, 27 and 28).

Group (b) *C. ovina* and *O. circumcincta* 4-12 days old and *N. spathiger* 1-3 days old:

Fewer worms were harvested from the Day 0 control (Sheep 33) than the Day + 2 controls (Sheep 34, 35 and 36). Most of the *C. ovina* in the last group were fourth stage larvae, which were present in uniform numbers (204-306). The number of *N. spathiger* present varied considerably and the distribution between the stages was uneven. The poorest recoveries were those of *O. circumcincta* (Table 4). There were, however, large numbers of *T. colubriformis* present, mainly in the fourth and fifth stage (Table 5). Their presence was probably due to contaminated cultures as the morphology of infective larvae of *T. colubriformis* and *O. circumcincta* is similar.

Group (c) *C. ovina* 12-26 days old and *N. spathiger* 4-12 days old

The numbers of *C. ovina* in the fourth stage, fourth moult and early fifth stage were very uniform both in Day 0 and Day + 1 controls.

One Day 0 control (Sheep 41) had a low worm burden, otherwise reasonably uniform numbers of *N. spathiger*, ranging from fourth stage to adult worms, were recovered from the controls.

Treated Sheep: Pyrantel tartrate at 25 mg/kg was highly effective against all stages of development. In those cases where its efficacy apparently fell to 61.5 per cent against the fourth moult of *C. ovina* and 69.2 per cent against the fifth stage of *O. circumcincta*, the results should be treated with reserve because very few worms were present (Table 4).

Discussion

There are two methods at present in use for testing the efficacy of anthelmintics against immature nematodes.

The larval anthelmintic test has been evolved in this laboratory. Susceptible worm-free hosts are infested at regular intervals with infective larvae throughout the prepatent period before the anthelmintic is dosed[3,4,5,6,7].

A more popular method developed by Banks & Michel[8], is the administration of a single massive dose of larvae of one specieis and treatment

Table 5.—Experiment 2: *T. colubriformis* recovered post mortem

Group	Sheep No.	Stage of development				
		3 M	L 4	4 M	5	A
CONTROLS..... Day 0	33	20	115	46	186	0
Day +2	34	0	406	5	564	0
Day +2	35	0	382	98	343	100
Day +2	36	0	319	127	753	108
TREATED..... Day +2	37	0	16	3	6	3
Day +2	38	0	6	4	10	0
Day +3	39	0	16	3	93	8
Day +3	40	0	3	0	0	0
Average Reduction.....		100.0%	96.6%	96.4%	94.1%	94.7%

of selected animals on specific days after infestation[2,6,9,10]. Gibson[11] has refined this rather empirical method by testing the anthelmintic specifically against the parasitic third stage, fourth stage and mature worms.

These experiments were an attempt to combine the advantages of both methods. Animals were repeatedly infested so that one group contained only third stage worms up to the end of the third moult while the others contained fourth stage, fourth moult and early fifth stage worms.

1. Third and fourth moults

To enable these experiments to be carried out it was essential to know the days on which moults occur. These are summarized in Table 6.

TABLE 6.—DAYS ON WHICH MOULTS OCCUR.

Species	Third Moult	Fourth Moult	Author
H. contortus	1½	9–11	VEGLIA[12]
T. colubriformis	3–4	8–10	MÖNNIG[13]
,,	3	6–10	DOUVRES[14]
O. circumcincta	2–3	7– 8	THRELKELD[15]
,,	3–4	Up to 84	SOMMERVILLE[16]
N. spathiger	<5	10–12	KATES & TURNER[17]
O. columbianum	4–5	26–30	VEGLIA[18]
C. ovina	<3	23–25	THRELKELD[19]

Although infestations with a single species are desirable, enough susceptible worm-free sheep were not available and mixed infestations had to be used. As a result the average days on which moults occurred had to be taken into account. By the third and twelfth day the third and fourth moult respectively has taken place for worms with a short prepatent period, i.e. the first four species listed (Table 6). The species *C. ovina* and *O. columbianum* complete the fourth moult from 23-30 days after infestation; 26 days was taken as the average.

With the exception of *O. columbianum* all species reached their third moult by the third day, therefore worms one to three days old covered the third stage and third moult. The fourth stage, fourth moult and early fifth stage were covered by the period of 4-12 days in *H. contortus, T. colubriformis, O. circumcincta* and *N. spathiger*. The period 12-26 days covered the latter half of the fourth stage, fourth moult and early fifth stage for *C. ovina* and *O. columbianum*.

2. Slaughter of experimental sheep

Controls: To determine the stage of development at the time of treatment controls were slaughtered either on Day 0 or within two days (Day + 2).

(a) One to three-day old worms

This was not completely successful, for two reasons. First, in spite of including ingesta of the fore-stomachs in the examination of Day 0 controls, and using Shone's waterbath[4], worm burdens in these controls were smaller than those in Day + 2 controls. Secondly, many worms had developed from the third stage on Day 0 to the fourth stage by Day + 2.

For worms in this age group only the Day 0 controls should be examined to determine the stage of development at the time of treatment and to confirm larval viability. The total worm burdens of Day + 2 controls are more reliable indicators of the worms present but not of the stage of development at treatment. The Day + 2 controls should therefore be used to estimate anthelmintic efficacy in comparison with the treated animals, the whole group being referred to as one to three day-old worms.

(b) Four to twelve-day old worms

With the exception of the low worm burdens in Sheep 33 (*C. ovina*) and of Sheep 41 (*N. spathiger*) the number of worms recovered from the controls was similar. Moreover, most of the worms were fourth stage larvae, regardless of the day of slaughter. These sheep could be killed on the day of treatment (Day 0) and their worm burdens compared with treated sheep killed two or three days later.

(c) Twelve to twenty-six-day old worms

Controls killed on Day 0 or Day + 1 had uniform worm burdens and could be compared with treated sheep killed either on Day + 2 or Day + 3.

Treated sheep: Slaughter was delayed for three days after treatment to enable sheep to expel those *O. columbianum* larvae in the histotrophic phase which had been affected by the anthelmintic. This was not very successful because pyrantel tartrate at 25 mg/kg was only moderately effective against worms in the gut wall.

As mentioned earlier "one to three day-old worms" should be referred to as a group. This

is well illustrated for *O. columbianum*, where a difference of one day in the slaughter of controls and treated sheep gave an apparent anthelmintic effect of 51.3 per cent against the third stage. Only one Day + 2 control had two worms in the third moult and none in the fourth stage while all the treated sheep killed on Day + 3 had worms in both the third moult and fourth stage. Obviously these worms should be grouped with the third stage, from which they had recently developed. This will reduce the efficacy against the third to 39.0 per cent rather than 51.3 per cent as shown in Table 2.

The efficacy against *H. contortus* in this group varied from 86.1 to 100.0 per cent and against *T. colubriformis* from 99.6 to 100.0 per cent. For the same reasons these results in Table 2 are misleading for worms one to three days-old (Table 2 and 4).

The moulting stages of all these parasites are of short duration and, with the exception of *T. colubriformis* in six sheep and *H. contortus* in two sheep, the numbers never exceed 100. Anthelmintic efficacy against these stages which were only present in small numbers, even in the controls, should therefore be treated with reserve. There seems, therefore, to be no object in classifying "in moults" in anthelmintic tests.

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OMPHALO-ALLANTOIC SNARING CAUSING BOVINE EMBRYONAL PATHOLOGY: A CASE REPORT.

W. H. GERNEKE and H. P. A. DE BOOM.*

SUMMARY

Snaring of the horns of the allantois by limbs of the regressing yolk sac in a bovine foetus collected 31 days after insemination is described. The subsequent entanglement is held responsible for vascular compression causing microcephaly, cardiac dilatation and general erythroblastic congestion as well as regressive changes in the liver and mesonephros, and terminating in early death.

INTRODUCTION

Aberrations of the foetal membranes mechanically producing teratological effects in the conceptus have been recognized, amniotic adhesions and strangulation or even amputation of parts of the foetus by the umbilical cord being best known. In many of these instances doubts have been cast as to whether the aberration of the membranes or cord could be held responsible as the primary factor.

During a pilot survey of early bovine prenatal development we were fortunate in finding an abnormal 31-day old embryo, the abnormality and subsequent death of which could be ascribed without doubt to circulatory disturbances consequent upon snaring of the horns of the allantoic sac by the limbs of the yolk sac. No previous record of such an anomaly could be found. By their very nature, early embryonal deaths would not lend themselves to timely detection so that specimens would not be available for examination, unless by chance. The significance of this case is increased, furthermore, by the possibilities raised with regard to embryonal resorption in the cow.

HISTORY

A clinically healthy pedigree Jersey cow, 6 years, 11 months and 9 days old, which had given birth to five normal calves, had been artificially inseminated on 27th January, 1967 by sperm from "Rosignol", a registered Charolais bull of known fertility. Thirty-one days later the cow was slaughtered according to a programme of investigation of early bovine prenatal development. During this time, as before, feeding and husbandry had followed a standard routine pattern as practised at the Research Institute for Animal Husbandry of the Department of Agricultural Technical Services at Irene. Towards the latter part of the period she had developed foot-rot, and five days before slaughter had received 125 ml Sulphamethazine (33⅓% solution) plus 15 ml of penicillin (300,000 IU/ml) intravenously. Although routine rectal examinations had been scheduled, they had not been carried out on this case: a fortunate oversight in retrospect.

OBSERVATIONS

The left maternal ovary contained one large follicle caudally, several scattered smaller ones and had an overall weight of 4.734 g. The right ovary contained a large corpus luteum in the ventrocranial ridge, two medium sized and several smaller follicles elsewhere and weighed 10.90 g. The rosette was indistinct, flattened and covered irregularly by peritoneum. The cervix had one

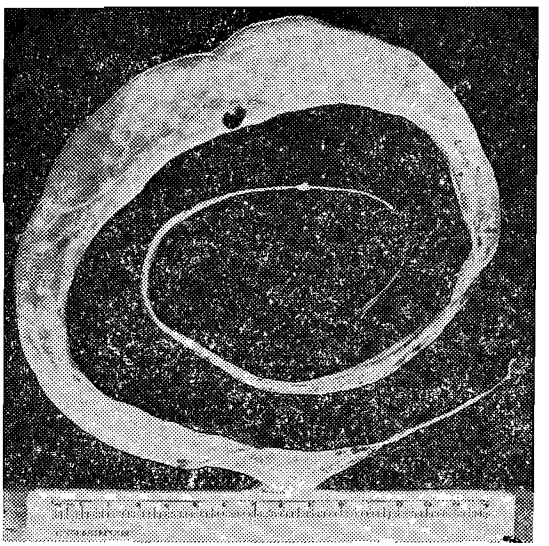

Fig. 1 — Chorionic sac before dissection revealing abnormal allantoic bulges and degenerated dead embryo of 31 days.

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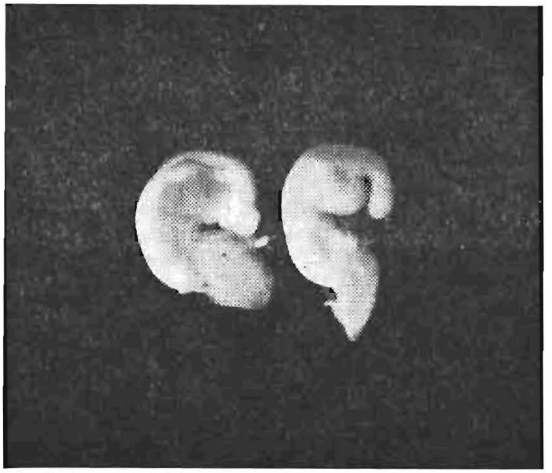

Fig. 2 — Degenerated, dead embryo of 31 days old on the right compared to a normal one of approximately the same age on the left. The microcephaly, cardiac dilation and liver atrophy are distinctly seen.

fold prolapsed and was completely closed, allowing only the tip of the small finger to enter. It contained tough, sticky, light brown mucus. The vaginal mucosa was moist and covered cranially by a small amount of mucus.

The horns of the uterus were moderately distended, the right horn, with a maximum circumference of 14.1 cm being slightly more so than the left horn (13.1 cm) and over a greater extent. The chorion occupied the lumen of the right horn completely up to 4 cm from its most cranial end (fig. 4), where the one necrotic tip could be picked up. The chorion also extended into the caudal end of the left horn just beyond the cornual junction to the body of the uterus. It was lightly attached to the endometrium by minute villi which were slightly more distinct over the cotyledons. The latter were better developed in the pregnant than non-pregnant horn. The allantois did not fill the chorionic sac, being restricted to a central area of 18.5 cm. Irregular constrictions of the allantoic sac visible through the chorion further attracted attention. Upon dissection it became clear that the left allantoic horn (Fig. 3 L_2) had become snared by the right limb of the filamentous yolk sac, thus causing a

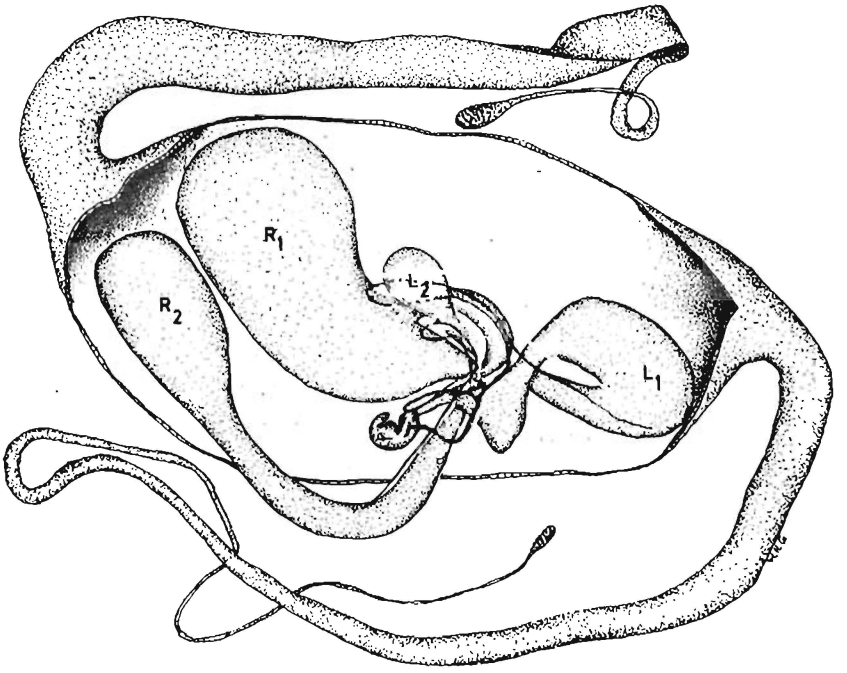

Fig. 3 — Sketch of the dissected chorionic sac revealing the right and left horns (R. & L. resp.) of the allantois and the manner of ensnaring of these by the horns of the yolk sac (in red).

looped left horn. This in turn had apparently caused the right allantoic horn (R) to form a loop around this ensnared tip with its apex (R_2) extending into the right chorionic horn via its own bifurcation. Due to the constriction produced at this bifurcation by the tangled limbs of yolk sac and allantois, the right limb of the latter had developed an additional horn (R_1) while the left horn had also started to bulge (L_1). The allantois was thus prevented from extending normally into the chorionic sac. Separation of the allantois from the chorion was easily performed in 70% alcohol after fixation in Bouin's fluid. The membranes could be drawn apart with due care as the mesoderm had not yet formed a very firm union.

The amnion (omitted in fig. 3) closely invested the embryo as would be expected at this stage of development. It had not been involved in the abnormal manoeuvres of yolk sac and allantois.

The embryo had undergone considerable pathological changes consequent upon interruption of its vascular supply from the allantois, as can be seen upon comparison with a normal embryo of approximately the same age (fig. 2). It had undoubtedly been dead at the time of slaughter of the mother and autolysis had already set in. In addition, necrobiotic changes had affected primarily those tissues with the highest metabolic rate. The heart had undergone marked hypertrophy and all vessels were much distended as a result of severe erythroblastic congestion. The liver sinusoids were greatly distended and as a result atrophy of liver cell cords with distinct focal karyorrhexis had become evident. Atrophy of mesonephric tubules, generalised hyperaemia and pycnosis of nuclei as well as poor development of the neural vesicles, which on sectioning revealed considerable autolysis and pycnosis, were amongst the more marked degenerative changes. The cranial ectoderm was infiltrated with neutrophils.

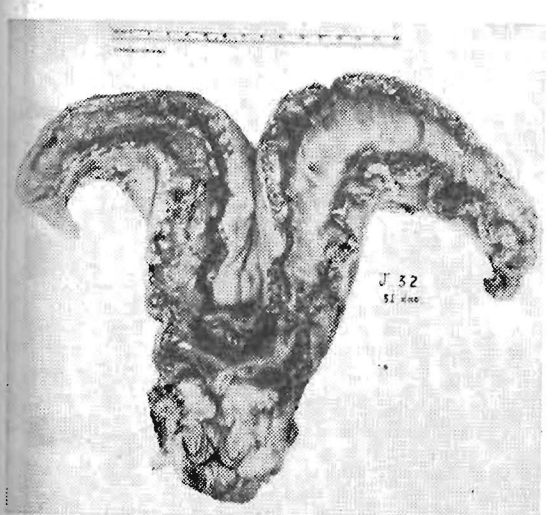

Fig. 4 — The chorionic sac in position in the right uterine horn.

The adrenals of the mother were peculiar in having a swollen and enlarged adrenal medulla covered by a thin cortical region. The total adrenal weight was 47.294 g. (L. 24.422, R. 22.872); almost double the normal weight. The pineal of the mother was also increased in weight.

Discussion

Initially the yolk sac grows out in the form of two limbs to form an inner lining for the chorion and at 22 days is still in contact with its ends[1]. It soon separates from the chorion, becomes threadlike and undergoes centripetal involution. The main allantoic bloodvessels are eventually seen lying parallel to and in the same position as these involuting yolk sac cords. During the earlier stages of involution of the yolk sac, before allantochorionic union is firmly established, its limbs are loosely arranged in the chorion and could easily become entangled with the outgrowing allantoic horns. In the case described it can be speculated that such entanglement must have overtaken the allantois and yolk sac about 6-8 days prior to slaughtering. The increase of fluid in the allantois in its abnormal position would cause a progressively greater constriction of the bloodvessels in the region of the umbilicus — resulting in cardiac hypertrophy; congestion; cephalic, liver and kidney atrophy and eventual early death of the embryo. The final outcome would be resorption.

From the pathogenesis outlined above, it is clear that only in those species such as ruminants and pigs, characterised by early and enormous elongation of the foetal membranes would omphalo-allantoic snaring and strangulation be a distinct hazard. The concept that severe teratological changes are responsible for many early embryonal deaths has gained general acceptance. It is in this light that such a hazard assumes particular significance.

As the embryo already was 1.5 cm long, the resorption would probably extend over a fair period of time. This would prevent the animal from coming on heat, with the result that one, possibly two, heat periods would be missed without revealing any clinical symptoms as to the cause. In the horse, van Niekerk[2] found resorbing

foetuses to terminate as pellets of necrotic tissue, which he believed suppress oestrum for as long as they exist in the uterus: their removal by saline flushing hastened the onset of the next heat period.

Rowson & Dott[3] stated that amniotic palpation could result in foetal death due to foetal heart rupture. It is conceivable that handling of the uterus could produce, amongst other effects, entanglement of allantois and yolk sac, yet, in this particular case, no palpation had been undertaken.

An imposed or inherently irregular or asymmetrical growth rate in the walls of each of the two membranes would produce deviations from the normal direction of growth and so could lead to entanglement.

The only abnormal external conditions operative in this case were the infection of the feet by *Actinobacillus necrophorus* and the treatment thereof. On the face of it one has no reason to incriminate these factors nor to exonerate them entirely. The significance of the enlarged medulla of the maternal adrenals is unknown.

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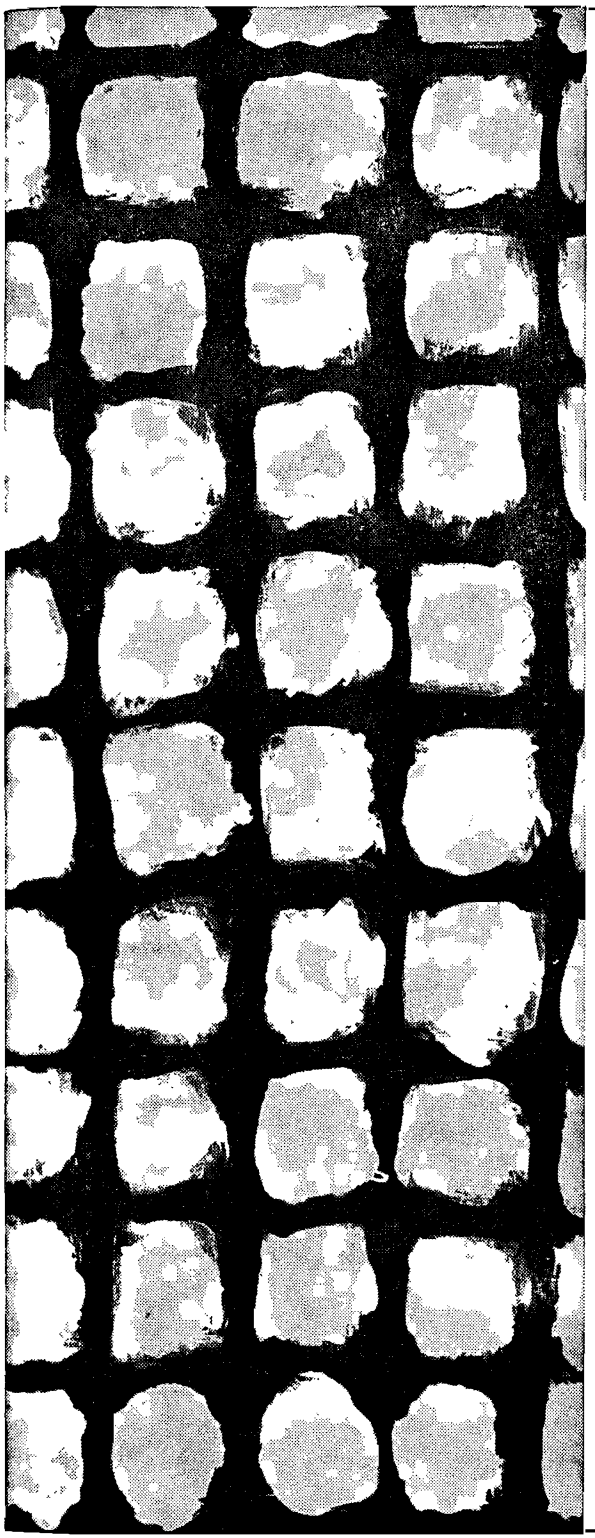

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KARYOTYPE OF *CERATOTHERIUM SIMUM SIMUM* AND *EQUUS ZEBRA ZEBRA*: A PRELIMINARY NOTE.

IRMGARD G. HEINICHEN*.

As part of a cytological survey on members of the order Perissodactyla preliminary observations on the karyotype of the White Rhinoceros, *Ceratotherium simum simum* Burchell, 1817 and the Cape Mountain zebra, *Equus zebra zebra* Linn., 1758 are recorded. The results were obtained by studying spreads prepared from bonemarrow biopsies taken while the animals were chemically immobilized.

The chromosome number of the White Rhinoceros was found to be 2n = 82. This figure is based on examination of 33 good spreads pre-

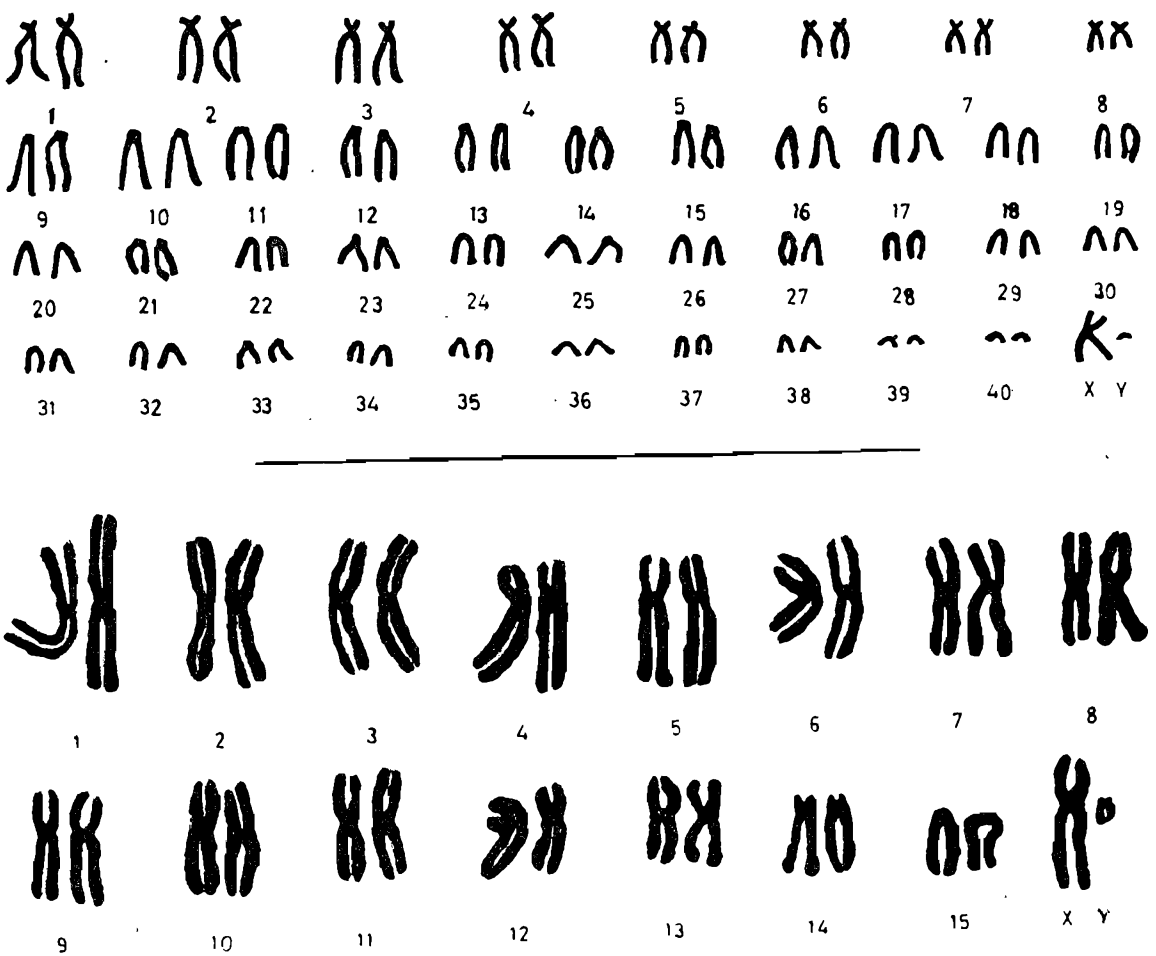

Fig. (Top) Karyotype of *Ceratotherium simum simum* (A Camera Lucida drawing).
(Bottom) Karyotype of *Equus zebra zebra* (A touched-up photo).

* University of Pretoria: Dept. of Anatomy, Faculty of Veterinary Science and Dept. of Genetics, Faculty of Agriculture

pared from one male and a female and is supported by counts on leukocyte cultures[1]. This is the highest figure yet obtained for the diploid chromosome number of any mammal, that of *Tarsius bancanus* (2n = 80)[2] and that of the dog (2n = 78)[3,4] being second and third in line respectively.

The karyotype of the White Rhinoceros (upper fig.) shows group I to consist of 8 submetacentric pairs and group II of 32 acrocentric chromosome pairs with a large metacentric X- and a small acrocentric Y-chromosome.

The Mountain Zebra was found to possess the diploid number of 32 chromosomes. This number was present in 128 out of 150 spreads. This figure differs from the number 34 inferred by Benirschke[5] for this animal. The karyotype of the Mountain Zebra (lower fig.) consists of one group of 13 pairs ranging from meta- to submetacentric chromosomes and a second group containing one telocentric and one acrocentric pair plus a metacentric X- and a submetacentric Y-chromosome.

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FIRST INTERNATIONAL PIG VETERINARY SOCIETY CONGRESS

The First Congress of the International Pig Veterinary Society will be held at Cambridge, England, from 17th - 21st June, 1968. It will include papers dealing with a wide variety of disease conditions in pigs and there will also be a full programme of visits to nearby research institutes and places of interest in the area.

Early application for the Congress is advisable and should be made to the Secretary, Mr. A. R. M. Kidd, Central Veterinary Laboratory, New Haw, Weybridge, England.

THE ACTIVITY OF TETRAMISOLE IN GOATS.

J. L. PRETORIUS*, and W. T. HARROW*.

SUMMARY

Critical anthelmintic tests of Tetramisole carried out in goats gave results comparable to those obtained by other workers in sheep. They also confirmed the brief results in goats published by Walley[1] and by Fitzsimmons[2].

Whereas a dose rate of 6 mg/kg was highly effective against all stages of *Trichostrongylus* and *Haemonchus* and adult forms of *Ostertagia, Gaigeria* and *Chabertia,* 15 mg/kg was required for the adequate removal of all stages of all these parasites.

Comparatively small-scale toxicity trials indicated that goats may be less susceptible than sheep to the side effects produced by overdosage.

INTRODUCTION

Although there is now extensive literature on the activity of Tetramisole in cattle and sheep there is, apart from Walley[1] and Fitzsimmons[2], no published work on its effectiveness in goats.

The present work is based on the techniques for the testing of anthelmintics described by Reinecke and others[3,4,5], and demonstrates the efficacy of the drug against fourth, fifth and adult stages of *Haemonchus contortus, Trichostrongylus spp., Oesophagostomum columbianum, Ostertagia circumcincta, Gaigeria pachyscelis,* and *Chabertia ovina.*

No work on the toxicity of Tetramisole in goats has yet been published; Philip and Shone[6] treated 2,563 angora goats without loss.

MATERIAL AND METHODS

(i) Tetramisole was administered as "Tramisol" (I.C.I.) — a 3% solution of the active agent.

(ii) A mixed group of cross-bred Boer and Angora goats was given a dose of 30 mg/kg of Tetramisole to remove existing worms and was then divided into two sub-groups of twelve and thirteen animals respectively.

(iii) Sub-group I was infected with *Haemonchus contortus, Trichostrongylus spp.* and *Oesophagostomum columbianum* as shown in Table 1, and sub-group II with *Ostertagia circumcincta, Gaigeria pachyscelis* (percutaneously), and *Chabertia ovina,* as shown in Table 2.

TABLE 1.—EXPERIMENTAL DESIGN.

Day No.	Number of infective larvae dosed to each sheep.		
	H. contortus.	*Trich. spp.*	*O. columb.*
—49	—	—	115
—39	—	—	33
—30	—	—	30
—26	530	600	79
—12	640	600	—
—5	555	540	46

TABLE 2.—EXPERIMENTAL DESIGN.

Day No.	Number of infective larvae dosed to each sheep.		
	O. circum.	*Gaigeria*	*C. ovis.*
—63	—	120	—
—32	375	—	25
—20	420	—	37
—13	396	—	63
—6	75	—	90

(iv) On day 0, three goats in each sub-group were dosed with 6 mg, and four with 15 mg of Tetramisole per kg respectively.

(v) On day 7 all goats were slaughtered after starvation for 48 hours.

(vi) Post-mortem procedures were essentially those described by Reinecke.

(vii) For the sake of expediency the different stages of *Trichostrongylus* were counted together. Even at the lower dosage, removal of all stages was virtually complete.

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Results: Anthelmintic Effect.

These are summarised in Tables 3 and 4.

Controls.

The use of treated animals rather than animals reared worm-free is open to criticism, yet it will be noted that the worm burden of the untreated controls was reasonably uniform except in the case of *Gaigeria* and *Chabertia*. However, since the goats were obtained from highveld summer rainfall areas, it seems unlikely that the poor "take" of these parasites in some individuals was due to residual resistance. In any case the number of worms remaining in treated animals was significantly lower than that in the controls.

Treatment at 6 mg Tetramisole per kg

This dose rate showed very low efficacy (22.2%) against fifth-stage *Oesophagostomum* and fourth-stage *Ostertagia* (10%) and the "take" of fifth and adult stage *Chabertia* in the controls was insufficient to give statistical significance. At this low level, however, the drug was 73.4% effective against adult *Oesophagostomum*, 90% against adult *Ostertagia* and 95%, 99% and 100% effective against adult *Haemonchus, Trichostrongylus* and *Gaigeria* respectively.

Treatment at 15 mg Tetramisole per kg

At this, the recommended dosage, efficacy against immature *Oesophagostomum* rose to over 70% and against immature *Ostertagia* to 90%. Anomalously, the higher dose in this experiment removed fewer adult *Ostertagia* (80%) than the lower dose (90%). Apart from this, 95% to 100% of adult worms were removed.

Toxicity.

Seventy five goats were given 30 mg of Tetramisole per kg, and none showed any side effects apart from slight trembling in one or two. Forty seven of these, which were pedigree angoras, were given a double dose of "Lintex"* and vaccinated against bluetongue and enterotoxaemia at the same time.

A further 10 goats were given 45 mg/kg. Of these, six pedigree angora ram kids, grazing on good quality, irrigated lucerne, staggered slightly within 10 minutes of dosing but this effect had completely worn off within 20 hours. One Boer ram was completely unaffected by the triple dose and was able to jump on and off a ledge four feet from the ground without difficulty or any sign of incoordination.

Other goats receiving 45 mg/kg licked their lips and shook their heads more than usual about 10 minutes after dosing but were otherwise normal and these slight signs disappeared within 24 hours.

Definite conclusions from such a small number of animals are impossible, but it appears that goats may be less susceptible than sheep and cattle to over-dosage. Furthermore, it may be said that, as in cattle (Forsyth[7]), animals in good condition show side effects more readily than poorer ones such as those clinically affected by helminthiasis.

TABLE 3

SPECIES.		*H. contortus.*				*Trich. spp.*	*O. columb.*				*Trichuris*
Group	Sheep No.	Stage 4	Stage 5	Stage A	Total	Total.	Stage 4	Stage 5	Stage A	Total	Total
Control	27	100	205	435	740	293	37	10	5	52	10
„	29	115	285	210	610	795	11	5	10	26	0
„	39	90	310	615	1015	969	32	29	21	82	0
„	40	95	335	1090	1520	570	38	8	41	87	0
„	42	145	190	370	705	225	81	37	55	173	0
Average total		109	265	544	918	570	39.8	18	26.4	85	2
6 mg/kg	34	18	20	12	50	15	10	5	2	17	0
„	38	0	0	0	0	16	13	6	8	27	0
„	46	0	0	0	0	10	10	30	11	51	0
Average total		6	7	4	17	12	11	14	7	31.7	0
% reduction		94.5	95	95	95	99	72.4	22.2	73.4	62.7	N.S.
15 mg/kg	28	10	0	10	7	0	0	0	0	0	0
„	31	0	0	0	0	0	16	0	0	16	0
„	32	0	0	0	0	0	15	10	3	20	0
„	45	0	0	0	0	0	10	10	1	20	2
Average total		2	0	2	2	0	11.5	5	1	14	0
% reduction		99	99	99	99	100	71.1	72.2	96.2	79.7	N.S.

(Where the figure 10 appears the actual number is 10 *or less*)

* Trade-mark of Bayer, Leverkusen.

TABLE 4

Species / Group	Sheep No.	*Ostertagia* Stage 4	*Ostertagia* Stage 5	*Ostertagia* Stage A	*Ostertagia* Total	*Gaigeria.* Total	*Chabertia.* Stage 4	*Chabertia.* Stage 5	*Chabertia.* Stage A	*Chabertia.* Total
Control	17	25	48	58	131	1	14	2	0	16
	18	32	43	45	120	7	9	0	0	9
	20	110	305	215	630	3	8	1	5	14
	24	0	45	170	215	20	14	2	1	17
	25	35	115	295	445	0	12	2	0	14
	26	88	120	175	383	0	8	4	2	14
Average total		48	113	159	321	5	11	2	1	14
6 mg/kg	27	80	32	40	152	0	0	0	0	0
,,	28	42	20	0	62	0	0	2	0	2
,,	44	0	94	9	104	0	0	3	2	5
Average total		41	48	17	106	0	0	2	2	2
% reduction		10	50	90	60	100	100	N.S.	N.S.	80
15 mg/kg	21	0	17	80	97	0	0	0	0	0
,,	22	0	0	0	0	0	0	0	0	0
,,	23	10	10	10	30	0	0	0	0	0
,,	29	10	0	10	10	0	3	0	0	3
Average total		10	6	25	140	0	0	0	0	0
% reduction		90	90	80	80	100	90	100	100	100

(Where the figure 10 appears the actual number is 10 *or less*)

ACKNOWLEDGEMENTS

We have to thank Mr. P. J. Lewis for considerable technical assistance and Messrs. I.C.I. South Africa (Pharmaceuticals) Limited for supplies of "Tramisol".

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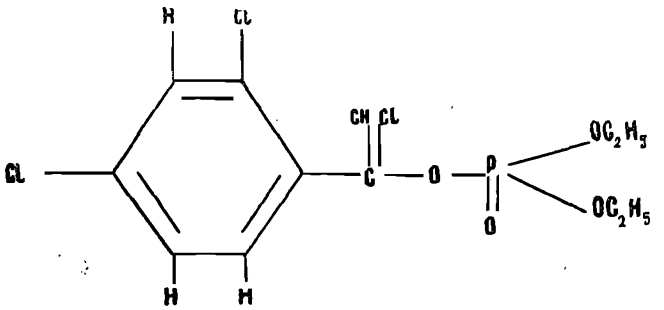

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A REPORT ON A STUDY OF VARIOUS ASPECTS OF EUROPEAN MEAT HYGIENE AND ABATTOIR BYPRODUCTS.

W. HOBBS*.

SUMMARY

A detailed report is furnished concerning various meat hygiene practices and procedures encountered during a tour of Denmark, The Netherlands and England. The study was made possible by the award of a travelling Fellowship. Details are provided of bacteriological, biochemical and biophysical laboratory procedures used as aids to meat inspection and the exercise of control over abattoir hygiene. The processing of abattoir byproducts was also investigated.

INTRODUCTION

Meat hygiene is becoming of even greater importance in all parts of the world, and the emergence of a Common European Market has emphasized the necessity of high standards and uniformity of approach in the handling and processing of the food animal. Having been awarded a post graduate Fellowship by the South African Livestock and Meat Industries Control Board for the study of certain aspects of meat hygiene, the writer proceeded to the Netherlands, Denmark and England for a period of six weeks. The following specific subjects were studied:

A. Laboratory procedures used as aids to meat inspection and hygiene control. These included bacteriological, biochemical and biophysical methods.

B. Abattoir hygiene.

C. The processing of abattoir by-products.

These three sections are covered under the heading of each of the countries visited, and the report is conduded with a discussion and some recommendations that the author considers worthy of introduction or further investigation in South Africa.

A. LABORATORY PROCEDURES USED AS AIDS TO MEAT INSPECTION AND HYGIENE CONTROL.

Introduction.

The laboratory plays an important part in most European countries, not only as an aid in meat inspection work but also as a control for abattoir hygiene and also in food handling establishments such as butcheries, restaurants etc. It is almost generally accepted that improvement in meat hygiene goes hand in hand with the use of the laboratory for the examination of meat and meat products.

Laboratory procedures also provide valuable corroborative evidence to justify either acceptance or rejection of carcases detained at visual inspection. Judgement of suspect carcases, based only on gross visible lesions and appearance, must of necessity be strict to avoid the danger of releasing potentially unsafe meat for consumption, but when the evidence of laboratory tests can be taken into consideration together with the organoleptic findings, the unnecessary condemnation of carcases is minimized. That this is true is borne out by figures quoted by Professor A. Jepsen regarding specimens examined bacteriologically at a Danish laboratory:—

	Number of suspect carcases	*Bacteriologically satisfactory.*
Cattle	765	92.8%
Pigs	258	78.4%

In both Holland and Denmark every abattoir of any size has its own laboratory whereas smaller abattoirs are required to send specimens for testing to specified central laboratories. These two countries have both municipally and co-operatively owned abattoirs. Meat inspection and laboratory work is done by municipal staff in the case of municipal abattoirs, and by state personnel in the case of co-operative abattoirs. Such laboratories employ one or more technical assistants who work under the direction of veterinarians.

Meat inspection in Holland is done by lay meat inspectors under very strict veterinary control e.g. Utrecht, population 270,000, employs a Veterinary Director of its abattoir, three other veterinarians and seven meat inspectors; in addition three meat inspectors are employed for the regular inspection of meat-handling concerns in the city. In Denmark even greater importance is attached to this work, and all meat inspection is done by veterinarians; and it is necessary that a veterinarian be employed full-time at any plant preparing canned meat

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products. Such a high degree of veterinary control must lead to greatly improved standards of meat hygiene, but is only possible where relatively large numbers of veterinarians are available for this type of employment. Apparently in both Holland and Denmark some 25% to 30% of all veterinarians are employed in meat hygiene work.

The position is very different in England where only a very few abattoirs employ veterinarians, and meat inspection is done by lay inspectors, generally without any veterinary control. Thus laboratory facilities are virtually non-existent at abattoirs although specimens for examination may be sent to any of the many regional veterinary diagnostic laboratories. However, there is no obligation on the inspector to take specimens for laboratory examination and there is no uniform procedure for such laboratory examination as is the case in Holland and Denmark.

Laboratory Procedures — Holland

Before describing the various laboratory procedures in use it will be of assistance to the reader to know in which instances these tests are performed. The following table indicates that in Holland carcases are classified, for meat inspection purposes, into five classes viz. A, B, C, D. Only carcases from groups A, B and D may be passed unconditionally and then only if the animal is normal at both ante-mortem and post-mortem inspection. If any abnormality is found, the bacteriological examination, pH and the boiling and frying test must be satisfactory.

1. *Bacteriological examinations of carcase specimens.*

Specimens required under Dutch law are:—

1. Whole spleen
2. One kidney
3. Muscle (*M. triceps* or *M. sartorius*), piece 8x8x6cm minimum.
4. Large piece of liver plus gall bladder.

It is desirable that specimens be taken to the laboratory as soon after slaughter as possible.

Media required:—

1. Meat agar, slopes (x4)
2. Glucose broth, tubes (x4)
3. Brilliant green/phenol red agar, plates (x2)
4. Sodium tetrathionate, large tube (x1)

Procedure.

Naturally the surface of each specimen is likely to be contaminated with micro-organisms and it is therefore necessary that the surface be sterilised before incising. This is done by burning the surface with a strong flame or by searing with a red-hot iron. The organ is then incised, using a sterile knife, and a gram or two of material is obtained from the depths of the specimen for inoculation of culture media.

Samples from each specimen are streaked on a meat agar slope and are put into a glucose broth tube. These are both non-selective media. In addition scrapings of liver substance and gall bladder mucosa are streaked onto separate plates of Brilliant green/phenol red agar. Finally a "cocktail" is made in which composite scrapings from each specimen are put into a flask of Na-tetrathionate broth, a selective enrichment medium for isolating *Salmonella*.

Following 24 hours incubation at 37°C, all cultures are examined for growth, and in addition a subculture is made from the Na-tetrathionate "cocktail" onto Brilliant green/phenol red agar. After a further 24 hours incubation all tubes and plates are again examined. If considered necessary, Gram-stained smears or hanging drops

Groups	A Normal at ante mortem inspection	B Casualty slaughter due to accident	C Emergency due to illness— no antemortem inspection.	D Animal Sick, slaughtered in presence of veterinarian	E Dead Animals
Bacteriological Examination, pH, Boiling/F Test	If required by veterinarian	Essential	Essential	Essential	Essential, provided carcase is fresh
Possible Disposal of Carcase.	1. Pass U. 2. Pass V 3. Pass S. 4. Pass F. 5. Condemnation	1. Pass U. 2. Pass V. 3. Pass S. 4. Pass F. 5. Condemnation	1. Pass V. 2. Pass S. 3. Condemnation	1. Pass U. 2. Pass V. 3. Pass S. 4. Pass F. 5. Condemnation	1. Pass V. 2. Pass S. 3. Condemnation

U — unconditional passing
V — passed only for Vrijbank sale (under supervision)
S — passed subject to sterilisation (under supervision)
F — passed conditional to freezing (cysticercosis control)

are examined microscopically. Should any of the Brilliant green/phenol red agar plates show a colour change to intense red a *Salmonella* may be involved, although certain *Proteus* colonies may cause the same change. Yellow colonies on this medium will be coliform bacilli. *Proteus* can be distinquished from *Salmonella* in several ways e.g. by characteristic growth on Triple Sugar Iron agar or on Decarboxylase medium or by carrying out the urease test. In most cases of suspected *Salmonella*, however, a slide agglutination test is carried out using polyvalent serum, and should this test be positive the culture is sent to the National Institute for Public Health for typing.

Interpretation of bacteriological findings. A "positive" bacteriological finding is made in the following cases only:—

(1) When either of the media containing muscle tissue or spleen show growth of whatever kind.
(2) When salmonellae are found in the kidney specimen.

The one exception to the above interpretation is that when only *Erysipelothrix insidiosa* is found in the media containing muscle and/or spleen, this is *not* considered as "positive" and the carcase may be passed conditional to sterilisation under supervision, providing it is sound in all other respects.

It is important to note that in Holland it is not necessary to make a diagnosis of the species of micro-organism present in any of the cultures, other than *Salmonella* and *E. insidiosa.* This, together with the clearcut definition of what constitutes a "positive" bacteriological examination, makes the interpretation simple and means uniformity of interpretation throughout the country.

In all instances of a "positive" bacteriological finding, the carcase concerned is condemned.

2. *Boiling and frying test.*

Every carcase that is detained for bacteriological examination has also to pass the boiling and frying test if outright condemnation is to be avoided. In practice the test is only a frying test and is done to ensure that the meat does not have any unpleasant odour or taste. The test is simple and involves frying an ounce or two of muscle tissue from the carcase under test in a frying pan containing either a little bland oil or fat tissue from the same carcase. The cooked flesh is then examined for abnormal odours and flavours, which if present means that the carcase must be condemned for human consumption.

3. *Determination of muscle pH.*

That the ultimate pH of the musculature of a carcase is of great importance in meat hygiene is well known, and in both Holland and Denmark every detained carcase that is bacteriologically satisfactory has also to be shown to have a sufficiently low pH before it may be passed as fit for human consumption.

There are several methods of determining pH, but in Holland only the electrometric method is approved. A reading of the pH is done, at the earliest, 24 hours after the animal has been killed, and is carried out using a freshly cut surface of muscle tissue. Usually the sample of muscle used for the bacteriological examination is refrigerated for 24 hours and is then used for the pH determination. Should the pH value obtained cause some doubt it is advised that readings be taken from both the fore and hindquarters.

The following pH values of muscle are considered as borderline in Holland:—

Horses 6.0 - 6.1; Cattle and sheep 6.1 - 6.2; Pigs 6.3 - 6.4.

Any carcase with a pH higher than these values is condemned for human consumption, but may be used for animal feeding e.g. zoological animals.

4. *Bile pigment determination.*

All carcases that exhibit an abnormal yellow colour of fat and connective tissue are subjected to the Hijmans-van den Bergh test for the presence of bile pigments.

Method: One part of blood-free fat tissue is shaken in a flask with about two parts of methyl alcohol until a smooth suspension is obtained. Add 1 ml of reagent mixture to 4 ml of alcoholic extract, and a rose-red to red violet colour indicates bilirubin.

Reagent. A Sulphanilic acid 1 g
25% Hydrochloric acid 15 ml
make up to 1000 ml with distilled water

B ½% aqueous solution of $NaNo_2$ (solution should not be kept for more than 3 weeks).

Mix A and B just prior to use in proportion 25 ml : 0.75 ml.

5. *Examination for trichinae.*

Trichinosis has not been diagnosed in domestic pigs in the Netherlands since 1926, and consequently it is only at Amsterdam abattoir that trichinoscopic examination is fairly regularly undertaken.

It is well known that the use of the trichinoscope is very time consuming and laborious, and for this reason the digestion method is in commoner usage when it is considered necessary to examine certain pigs. For digestion the following fluid is constituted:

Distilled water	500 ml
25% hydrochloric acid	5 ml
pepsin (1:10,000)	5 g

The fluid should not be stored for more than a few days. 20 to 40 g finely cut muscle tissue is placed in an Erlenmeyer flask with 500 ml of digestion fluid, the mixture is held at 37°C and shaken every 15 minutes. After 4 to 24 hours it is filtered through a 0.5 mm guage sieve and the filtrate is allowed to stand for at least 2 hours to allow any trichinae to settle. The supernatant fluid is decanted and the sediment is spread on a slide and examined microscopically.

Work has also been done in Holland on the serodiagnosis of trichinosis and to a certain extent a slide agglutination test using an American antigen, is employed.

6. *Detection of antibiotics in urine of slaughter animals.*

About 1 ml of a *B. subtilis* broth culture is spread over the surface of an agar plate and 1 cm discs of filter paper, saturated in the urine of the animals to be tested, are pressed onto the surface of the agar. Incubate for 18 - 24 hours at 37°C and examine. A disc surrounded by a distinct zone of inhibition of bacterial growth indicates that the urine under test contains antibiotic, and thus it is reasonable to suppose that the flesh of the animal also contains antibiotic residue.

The carcase of any slaughter animal that is known to have been treated with antibiotic preparations, or is suspected of having been so treated within a few days of slaughter, is tested for the presence of residues in this way. Any carcase containing antibiotic residue is declared unfit for human consumption.

7. *Ultra-violet ray examination for cysticercosis.*

Cysticercosis is becoming an ever-increasing problem in the Netherlands, and, as an attempt to improve the detection rate of animals infested at meat inspection, limited use has been made of ultra-violet light examination. The method is not entirely practical as it involves moving the head, with incised masseter muscles, and the heart of each animal slaughtered into a dark-room for examination under an ultra-violet lamp. Cysticerci show characteristic fluorescence under U-V light. It has been found that not only does the use of this method detect infested carcases that have been passed by the meat inspector but more important, its use improves the percentage of infested carcases found at routine meat inspection by stimulating inspectors to examine carcases more closely.

8. *Histological examination.*

Histological methods are not employed to any extent as an aid to meat inspection, except in the sense that such methods are of great value in controlling the condition of meat in premises such as butcheries, restaurants, etc. When the meat inspector on his inspection rounds finds meat or meat products in an apparently unsound condition he brings a sample, in 10% formalin, to the laboratory. There a block of the tissue is frozen and sections cut to 10μ in thickness. These are put in a ¼% solution of Methylene Blue for 1 to 2 minutes, washed several times in clear water, floated onto a coverslip, inverted on a glass slide and examined microscopically.

The presence of bacterial 'nests', and any degree of autolysis of the tissue, is sufficient evidence of decomposition. This method is obviously also useful in detecting any adulteration of certain meat products with tissues from forbidden organs.

9. *Other laboratory examinations.*

There are certain other laboratory procedures in Holland that are prescribed but are not in daily use e.g. determination of water and blood content of meat ("compressorium" test), detection of hydrogen sulphide in decomposing meat using lead acetate, etc. However, most of these tests are well known, and their value in meat inspection is questionable in as much as the information gained is scarcely more objective than that obtained by organoleptic examination.

Laboratory Procedures — Denmark

1. *Bacteriological examination of suspect carcases.*

The Danish Ministry of Agriculture has laid down that when certain disease conditions or states are encountered at meat inspection, the carcass concerned must undergo bacteriological examination. Only if this examination is satisfactory may such carcasses be passed for human consumption. Bacteriological examination is required in the following instances:—

(i) Sick and emergency killed animals (except very recent accident cases).
"Sick" denotes presence of 'fever' and/or disturbance in general condition.

(ii) Infectious diseases that do not necessarily require condemnation.
There are two such diseases (a) recovered cases of swine erysipelas and (b) afebrile cases of strangles.

(iii) Chronic pericarditis.

(iv) Traumatic pericarditis.

(v) Chronic verrucose endocarditis.

(vi) Bronchopneumonia purulenta.

(vii) Subacute pneumonia in young animals.

(viii) Gastro-intestinal catarrh if a specific pathogen is suspected.

(ix) Peritonitis, not healed, no evidence of fever.

(x) Chronic metritis.
(xi) Retention of placenta.
(xii) Incomplete delivery.
(xiii) Prolapse, tortion or rupture of the uterus.
(xiv) Chronic purulent mastitis.
(xv) Osteomyelitis, not extensive enough to justify condemnation.
(xvi) Infected fractures and infected wounds.
(xvii) Arthritis, in only one joint, other than in suckling animals.
(xviii) Uncomplicated burns.

Naturally, the veterinary inspector may request a bacteriological examination of any carcass about which he requires additional evidence before arriving at a decision as to whether it should be passed or condemned for human consumption. Provided the bacteriological examination, which is uniform throughout the country, is satisfactory, the carcass may be unconditionally passed. There is no conditional passing of carcasses (other than the freezing of carcasses lightly affected with *Cysticercus bovis*) as there is in the Netherlands. The carcass of any animal treated with antibiotic or sulphonamide drugs within six days of slaughter must be condemned unless laboratory examination shows that no residue of the chemotherapeutic remains. Emergency slaughter cases have, by law, to be accompanied by a veterinary certificate stating full details of any chemotherapy applied.

Specimens required for the examination are:—
(a) Spleen (intact whenever possible).
(b) One kidney (only when antibiotic residue is suspected).
(c) Piece of liver (about 500g) taken from hilar region, together with portal lymph nodes and empty gall-bladder.
(d) Two intact lymph nodes of the musculature (prescapular, precrural, popliteal or ischiatic).
(e) Piece of muscle, at least 250g, covered by intact fascia, preferably from the extensors of the forearm.
(f) Any other organ or tissue deemed advisable to examine.

Samples must be taken as soon after killing as possible and if they have to be sent any distance to a laboratory they must be refrigerated, wrapped separately, boxed and shipped by the fastest possible means. The samples must be accompanied by a form giving the complete *ante-mortem* and *post-mortem* findings and also the number of identification. Detained carcasses are placed in refrigeration pending the outcome of the laboratory examination and a visual re-inspection.

Procedure.

Material is taken aseptically from the depths of the specimens removed from the carcass and inoculated onto the culture media according to the following plan:—

(a) *Muscle*
  1. *Blood agar plate* (for haemolytic organisms)
  2. *Iron sulphite tube* (black anaerobic colonies : *Clostridia*)
  3. *Bromthymolblue — lactose — saccharose agar plate* (blue colonies may be *Salmonella* — subinoculate onto *Triple-sugar Iron* medium).
(b) *Lymph Node* — same media as muscle tissue.
(c) *Lymph Node* — same media as muscle tissue.
(d) *Spleen* — same media as muscle tissue but in addition a tube of *Na-Tetrathionate broth* is inoculated. This is a *Salmonella* inrichment medium. After 24 hours incubation subinoculate onto *Brilliant green — lactose — saccharose — phenol red agar.*
(e) *Liver and gall-bladder*
  Tetrathionate broth tubes, incubate 24 hours, subinoculate *onto Brilliant green — lactose — saccharose — phenol red agar.*

Approximately lg of tissue is used in each case and the material from muscle, spleen and lymph nodes, which has been transferred to empty petri-dishes, is smeared against the bottom of the petri dish in order to disperse part of the tissue in the medium, while the bulk of the tissue block is left to be embedded in the medium. The melted agar is poured into these plates and allowed to solidify. The iron sulphite tubes must be inocculated while the medium is melted and cooled down to about 45°C.

All cultures are incubated at 37°C for 18-24 hours, when the first reading is taken. From the tetrathionate broth, a few drops are streaked on a brilliant green — lactose — saccharose — phenol red agar for the isolation of any *Salmonella.* Blue colonies on bromthymolblue — lactose — saccharose medium may be *Salmonella* and are subinoculated by stabbing and streaking a butt/slant of Triple-sugar Iron medium.

When readings of the agar-plates and iron-sulphite tubes are negative, a second reading must be made after a further 24 hours incubation, especially as species such as *Erysipelothrix* and *Corynebacterium pyogenes* are fairly slow-growing. Thus after 48 hours the following readings can be taken:—

(i) The four blood-agar plates are examined for growth and especially for colonies causing haemolysis.
(ii) The four iron-sulphite tubes are examined for the presence of anaerobic black colonies, in-

dicating the presence of *Clostridia*. Besides *Clostridia* only *Salmonella* and *Arizona* cause blackening in this medium.

(iii) Any triple-sugar iron cultures that have been made 24 hours previously from blue colonies on the bromthymolblue-lactose-saccharose agar are inspected. Typical *Salmonella* cause the butt to change to a yellow colour, often with the presence of gas indicated by a splitting of the agar, and the formation of hydrogen sulphide which causes a black deposit. Positive identification can be carried out by agglutination or fermentation tests.

(iv) The two brilliant green-lactose-saccharose-phenol red agar plates made from the tetrathionate broth enrichment containing spleen and liver and gall-bladder are examined. Red colonies on this medium must be regarded as possibly being *Salmonella* though some species of *Proteus* may also show red colonies. The quickest method of diagnosing *Proteus* is by the use of the urease test.

(v) As *E. insidiosa* and *C. pyogenes* may only multiply within the tissue block embedded in the blood agar, it is necessary, when no visible colonies are present in the surrounding medium, to make Gram-stained smears from the tissue blocks and examine these for the presence of these two organisms.

Interpretation of Bacteriological findings.

Bacteria found in bacteriological meat examination may be either pathogenic or a mixed flora of non-pathogenic organisms resembling the natural intestinal flora. The term "specific infection" covers all species regarded as specific pathogens, as follows:— haemolytic streptococci, haemolytic staphylococci, *Diplococcus lanceolatus, Pasteurella, Salmonella, Escherichia* in newly-born animals, *Bacillus anthracis, Erysipelothrix, Listeria* and *Corynebacterium pyogenes.*

The term "non-specific infection" covers species of non-pathogenic or only potentially pathogenic bacteria such as *Streptococcus viridans,* enterococci, *Escherichia* in grown animals, clostridia, *Bacillus subtilis-mesentericus* group and non-haemolytic staphylococci.

Carcasses are reported as falling into one of the following four classes:—

(a) Sterile.
(b) Specific infection.
(c) Low grade non-specific infection (growth from one sample only, excluding the liver sample).
(d) High-grade non-specific infection (growth from two or more samples, excluding the liver sample).

Results classified as (a) or (c) present no hindrance to the carcass being passed, whereas those under groups (b) or (d) necessitate condemnation of the carcass.

2. *Determination of Muscle pH.*

In practice only the electrometric method of ph-determination is used and this is usually carried out on the muscle specimen submitted for bacteriologiacl examination. In fact, as in Holland, pH determination has to be made on muscle from every detained carcass giving a satisfactory bacteriological test. Carcasses with a pH higher than the borderline may still be unconditionally passed after- re-inspection or may be passed only as fit for canning purposes.

The following pH values are considered as critical in Denmark:—

Cattle 6.2 Pigs 6.5

While pH-determination is obligatory on all suspect carcasses in Denmark, it would appear that less importance is attached to it than in Holland.

3. *Frying test.*

This test is only used on meat from old boars to check the presence of sex odour. The test is not used in conjunction with bacteriological examination of carcasses as in Holland.

4. *Detection of antibiotic residues in carcasses.*

When an animal is reported to have been treated with an antibiotic substance within six days of slaughter, or is suspected of having been treated, Danish legislation requires that the carcass be detained and a kidney be sent to one of four central laboratories to determine the presence or otherwise of any residue. Should antibiotic residue be present, the carcass is condemned.

For the test a slice of kidney is embedded in media containing a highly susceptible *Sarcinia lutea* culture, incubated overnight and examined for a zone of growth inhibition.

5. *Examination for Trichinae.*

Trichinosis is no longer a problem in Denmark and examination of all pigs is not considered necessary. However, a check is kept on the position by subjecting every pig carcass weighing more than 200 kg to trichinoscopic examination. In this way all older pigs that find their way to the slaughterhouse are screened. No use is made of agglutination or digestion methods of detecting infection and only the trichinoscope is used.

6. *Resazurin Reduction Test.*

This test is used in Denmark as a simple method of determining the hygienic quality of meat and meat products. It is based on the activity of enzymes produced by bacteria in the meat that are able to reduce the dye resazurin. The

principle of the test is the same as the methylene blue reduction test used in the quality control of milk. Results cannot necessarily be correlated with the results of bacterial counts done on the product concerned, because bacterial counts only take into consideration the number of live bacteria present, whereas enzymes that will reduce resazurin and similar dyes can be the products of bacteria already dead. The decomposing abilities of bacteria do not cease the moment these bacteria die but may continue until after the cell has disintegrated.

This test then does not replace bacterial counting techniques, but it does give a valuable indication of the hygienic quality of the product under test. Besides being of use in the meat factory, the test is a valuable aid in the re-inspection of carcasses that have perhaps been consigned over long distances and where some doubt exists as to whether or not decomposition is setting in. Furthermore, as the test can be performed by the unskilled it can prove a stimulus to meat factory workers to try and lengthen the reduction times and thus demonstrate an improvement in the hygienic quality of the products concerned.

Procedure. Resazurin paper is made by saturating filter paper in a 0.01% aqueous solution of resazurin and allowing to dry. The paper is then cut into pieces 2-3 sq inches in size, and is best stored wrapped in aluminium foil as the dye is affected by light. The test sample of meat need only be a thin slice of several square inches which is placed in a polyethylene bag. Composite samples can also be taken when very small samples are kneaded together in a bag so that the pieces mix well and their surfaces rub one another, thus ensuring an even distribution of bacteria. Frozen meat should be thawed prior to examination by this method.

Three pieces of resazurin paper are moistened in tap water and are placed on the meat in the plastic bag. Great care should be taken that good contact between paper and meat is obtained. The bag is turned so that the meat rests on the paper. After one minute the papers are removed and placed in a clean plastic bag from which the air is carefully expressed. The bag containing the test papers must be kept in the dark during the test and this is best accomplished by placing it in a desk drawer.

The colour of the strip is controlled after 10, 30 and 60 minutes. The whole colour range is from azure blue to violet, red-violet, clear rose red, and finally decoloration takes place.

Interpretation. Colour change to red or colourless:

in less than 10 minutes	: not acceptable
between 10 and 30 minutes	: just acceptable.
between 30 and 60 minutes	: good quality.
longer than 60 minutes	: excellent quality.

7. *Other laboratory procedures.*

The only other routine laboratory procedure connected with fresh meat inspection in Denmark is the microscopic examination of smears from all submaxilliary lymph nodes of pigs showing abscessation. Such smears are stained by the Ziehl-Nielsen method and examined under the microscope so that abscesses caused by *C. pyogenes* can be distinquished from lesions caused by tubercle bacilli. In all cases where mycobacteria are found, and also in the case of negative smears, the carcasses concerned are ineligible for export generally and to England in particular.

Laboratory Procedures — England.

Up to the present time laboratory procedures in relation to meat inspection have been largely neglected in England. None of the abattoirs visited have any laboratory facilities of their own, although none are very far distant from one or other regional veterinary laboratory, to which specimens from suspect carcasses may be sent if the meat inspector so wishes. Contrary to the situation in most western European countries, it is not compulsory in England for a bacteriological examination and pH determination to be carried out when certain conditions are found or are suspected of existing in a carcass. In consequence very few carcasses are ever detained pending further laboratory investigation. Furthermore, there is no uniform procedure of carrying out the laboratory examination of material from suspect carcasses, and each laboratory follows its own procedure. The procedures set out below are those used by the Liverpool Veterinary Laboratory.

1. *Bacteriological Examination of Carcass Specimens.*

Samples required are:—

(i) ½ - 1 lb skeletal muscle covered by intact fascia.

(ii) ½ - 1 lb liver together with a hepatic lymph node and the emptied gall bladder.

(iii) Whole spleen.

(iv) One kidney.

(v) Two intramuscular lymph nodes (prescapular, precrural, axillary or popliteal).

(vi) A piece of any other organ, the pathology of which makes examination desirable.

The culture media required are as follows:—

(i) Blood agar, 5%
(ii) Glucose agar, 1%
(iii) Selenite broth.
(iv) 10 per cent salt broth.
(v) 1/2000 solution of sodium azide in 0.5 per cent glucose broth (for detection of *E. insidiosa*)
(vi) MacConkey agar plates.

Procedure. About 1 g of material is removed aseptically from the depths of each specimen and is inoculated onto culture media according to the following chart:—

Muscle and two lymph nodes	Blood agar (pour plate technique) Glucose agar (shake cultures) Selenite broth 10% salt broth Sodium azide broth (pigs only)
Spleen, liver, kidney.	Blood agar (pour plate technique) Glucose agar (shake cultures) Selenite broth MacConkey agar (liver, gall-bladder and hepatic lymph node only)

Note: Composite scrapings of hepatic lymph node and gall bladder mucosa are inoculated into selenite broth, together with liver tissue, and also onto the surface of a MacConkey plate.

After 24 hours incubation at 37°C, subcultures are made from all fluid media and from tissue remaining embedded in the solid media when these fail to show signs of bacterial growth. All cultures are then incubated for a further 24 hours, and positive cultures identified after this time.

Interpretation of bacteriological findings. It is emphasized that the bacteriological methods provide only an aid to diagnosis and that the final judgement on a carcass remains the responsibility of the meat inspector concerned. However, the following standards are suggested:—

1. Evidence of recent bacteraemia, pathogenic organisms ..... condemn carcass
2. Evidence of recent bacteraemia with unusually large numbers of non-pathogenic bacteria ..... condemn carcass (e.g. colony counts over 9 per sample)
3. Other bacteriological results ..... release carcass

2. *Measurements of pH of Muscle.*

Either the Nitrazine-yellow indicator test or the electrometric method is used for the pH determination of muscle from suspect carcasses. However, there is no hard and fast rule as to what is acceptable as a pH reading and the measurement is taken merely to provide additional evidence. It is left to the inspector to make a careful reassessment of the carcass before coming to a final decision as to its disposal.

Discussion

1. *Bacteriological examination of suspect carcasses.*

Bacteriological methods are, without doubt, the greatest single laboratory aid available to the meat inspector, and while every abattoir laboratory could develop its own peculiar procedure in this regard it is considered that in any country the following features are highly desirable:—

(a) Uniform regulations laying down those cases in which bacteriological examination is essential.
(b) Uniformity of procedure — i.e. samples required, methods and media.
(c) Uniformity of interpretation.
(d) Simplicity of procedure and interpretation.
(e) Reliability of methods.
(f) Lowest possible cost.

In Holland, in certain instances, it is left to the veterinarian to decide whether to detain a carcass while a bacteriological examination is carried out or whether to pass (conditionally or unconditionally) or condemn the carcass without such examination. Of course, one must remember that in Holland judgement of a carcass is simplified due to the existence of the two avenues of conditional approval available (excluding freezer treatment of "measly" carcasses) viz. "Vrijbank" sale of fresh meat in small quantities to underpriviliged persons, and sterilisation of meat prior to its being sold. Where no provision is made for conditional approval of suspect or inferior quality carcasses, it is suggested that a list of conditions be compiled so that it would be obligatory for bacteriological proof of safety for human consumption to be obtained prior to approval of carcasses in which any of these conditions were found at meat inspection. Such is the position in Denmark.

That the methods used in subjecting a carcass to a bacteriological examination, and the interpretation of such examination, should be standardised throughout a country, goes without saying and needs no elaboration.

Furthermore, it is highly desirable that the procedure adopted should fulfil the conditions of reliability, simplicity, rapidity and should also be reasonably inexpensive. In this regard it is considered that the Dutch procedure fulfils all these requirements, but it is felt that a slight improvement could be made by including a shake culture of muscle specimen in an iron sulphite agar tube, as no provision is made for determining the presence or otherwise of anaerobes, especially clostridia. The use of blood agar, as in the Danish procedure, is of immense help in distinguishing between pathogenic and non-pathogenic organisms as the former, by and large, produce haemolytic colonies. However, the preparation of sterile blood agar is not easy, especially in the smaller laboratory, and the benefits of its inclusion as one of the media to be used in routine carcass examination work depend very largely on the way the bacteriological examination is interpreted.

The Danish procedure of bacteriological examination is ideally suited to the system employed in interpreting the results obtained by such examination, but is somewhat more elaborate than the Dutch procedure. Perhaps this is partly borne out by the fact that the Danish method requires 14 initial cultures (tubes and plates) to be made whereas only 11 are required by the Dutch method. It is felt, however, that the Dutch procedure would remain equally reliable were the four meat agar slopes excluded — surely any infection would be easily determined in the broth cultures alone. The procedure in use at Liverpool, England makes 24 initial cultures necessary.

The most important single factor involved in the bacteriological examination of a carcass, no matter what specimens or culture media are employed, is the interpretation of the results obtained. The entire object of employing laboratory aids in meat inspection work is obviously nullified if the interpretation of the results is not based on sound scientific thinking. Unfortunately, there are two schools of thought on this matter of the interpretation of the bacteriological findings of suspect carcasses, and both have some merit.

In the Second Report of the Joint FAO/WHO Expert committee on Meat Hygiene WHO Techn. rept. Series No. 241 (Geneva, 1962) it is stated that "the use of bacteriological methods in post-mortem inspection is based upon the theory that the internal organs without direct connection to the exterior, and muscular and lymphatic tissues of healthy animals, which were slaughtered while in a physiologically normal condition, are sterile. If bacteria can be cultivated by these methods, this indicates the possibility of an abnormal condition". However, the presence of non-pathogens in limited numbers in such tissues as spleen, lymph nodes and muscle, is not considered in Denmark as justifying condemnation of the carcass concerned. In Holland the presence of any bacterial infection in any of these tissues, whether pathogenic or non-pathogenic, whether few or in great numbers, is sufficient evidence to warrant condemnation of the carcass. Professor A. Jepsen of Copenhagen, explains the presence of non-pathogens (i.e. non-specific infection) in muscle tissue, spleen and lymph nodes of normal animals after slaughter in the following terms: "It has been mentioned that some instances may be explained as the result of infection through the sticking wound, but in most cases the bacteria are apparently of intestinal origin. Although the mechanism is not well understood, it seems an established fact that a breakdown of natural resistance may, in some way or other, lead to an invasion into the tissues of bacteria from the intestinal tract or from other naturally infected cavities, that subsequently spreads through lymph and blood vessels and results in bacteraemia. This is prone to happen in animals that are slaughtered when in a poor or exhausted condition due to systemic disease or physical strain".

Thus it would appear that, in some circles, the presence of non-pathogenic organisms in normally sterile tissues is considered to be an occurrence that can be associated with the slaughter process, and provided this infection is slight ("low-grade") this provides no reason for the condemnation of the carcass. My own view is that an unspecific infection of the musculature and/or the circulatory system (represented by the spleen), in itself of no danger to the consumer of the meat, favours spoilage and bacterial decomposition. The keeping quality of such a carcass must be affected to a very great extent, and especially so in a warm country such as South Africa, and particularly where carcasses are not chilled very promptly after the dressing process. Therefore it is felt that suspect carcasses showing any bacterial infection whatsoever in muscle or spleen samples should be condemned for human consumption. Similarly, the presence in any tissue of a suspect carcass of micro-organisms known to be pathogenic, must lead to the condemnation of that carcass. This method of interpretation is followed in Holland.

Several advantages are to be gained when results are interpreted in this way, viz.: —

(1) Judgement of results is generally very straight forward as no diagnosis of the type of micro-organism concerned is necessary. This is important in a small la-

boratory where manpower is limited and where there may be no specialist bacteriologist.

(2) The more simple or straight forward the interpretation of results is made, the more uniform will be the judgement of results at both the same laboratory and different laboratories.

(3) Very often muscle and spleen cultures can be shown to be positive after 24 hours incubation, which means the carcass can be condemned without further delay and without the necessity of making sub-cultures.

Figures taken out in Denmark on the results of some 7000 bacteriological examinations on suspect carcasses show the following results:—

Specific infection	12%
Non-specific, high grade	12%
Non-specific, low-grade	6%
Sterile	70%

Thus, approximately 76% of suspect carcasses are eventually passed as fit for consumption in Denmark.

Comparable figures taken from Utrecht abattoir in Holland for the year 1965 show that 82.8% of carcasses detained for bacteriological examination were finally passed:—

Suspect carcasses	1829	
Carcasses passed	1515	(82.8%)

It is necessary to realise that there may be other than bacterial reasons for condemnation of a carcass. Professor Jepsen puts it in the following words: "It must be clearly understood that bacterial examination has certain obvious limitations and can never become a substitute for organoleptic inspection; it is a supplementary measure only, to be used under specified conditions. It would be a technical error to apply bacterial examination when non-infectious pathological conditions call for condemnation of a carcass".

The cost of subjecting carcass specimens to a bacterial exmaination is not unimportant, and the question arises as to who is responsible for those costs. In Denmark the present charge for this service is equivalent to R2.85 per suspect carcass and is paid by the owner of the animal (farmer, butcher or wholesaler). The fee for carrying out an examination for antibiotic residue in a carcass is equivalent to R1.43.

2. *Other Laboratory Procedures.*

Most of the other laboratory methods used in the countries visited are in use in this country and therefore require no further comment. Of the remaining tests it is felt that the resazurin reduction test, giving useful information as it does and being quick and simple to perform, merits introduction in South Africa. The histological procedure used in Holland on meat and meat product samples is very useful in controlling hygiene, but possibly the resazurin test makes this superfluous.

It is considered that antibiotic residues in meat in South Africa, considering the farming methods used, will be found in a very limited number of carcasses only (poultry excluded). Should it be considered desirable to eliminate as many of the affected carcasses as possible, it would seem that legislation, such as exists in Denmark, would have to be introduced. In that country only veterinarians may administer antibiotic preparations to animals, and it is compulsory for all veterinarians to supply the slaughterhouse concerned with a specified certificate in the case of all animals that are slaughtered within six days of receiving an antibiotic drug. However, it is reasonable to assume that in South Africa milk plays a far bigger role than meat in conveying antibiotic residues to the human population.

Trichinosis has not been diagnosed in this country to date, but this is not to say that the condition does not exist. It is suggested that it would not be out of place for some of the larger abattoirs to occasionally examine the carcasses of old boars and sows for the presence of *Trichinae*, and either the digestion method or the slide agglutination test would be less laborious than the trichinoscopic method. Other than old pigs it might prove valuable if diaphragm samples from human autopsy subjects over the age of 40 years were examined by the same methods.

The use of the ultra-violet lamp to detect cysticerci in meat is impracticable as an abattoir routine, but experience in Holland does suggest that more attention should be paid, during meat inspection, to a thorough examination of the heart. Unfortunately the relevant regulations in this country do not include the examination of cut surfaces of the cardiac muscle, and it might be found, as is reasonable to expect, that the heart muscle is the site of prime importance for the location of cysticerci.

B. *ABATTOIR HYGIENE.*

The correlation between the keeping quality of meat and the degree of cleanliness during the slaughter process, and up to the time the meat reaches the consumer, is well known.

In South Africa, unfortunately, there is at the present time very little control over the conditions to which meat is subjected once it leaves the abattoir, and expecially in regard to the conditions under which it is transported. It is almost pointless to raise abattoir hygiene to perfection when the conditions under which the meat is subsequently handled leave so much to be desired.

However, legislation is at present under consideration which, when introduced, will serve to tighten-up considerably on the standard of post-abattoir meat hygiene. Those responsible for cleanliness within the abattoir must be sure that the abattoir itself does not then become the weakest link in the chain of the clean production and handling of a perishable article.

It is fairly obvious that it is impossible to produce a completely clean (sterile) carcass under practical abattoir conditions, just as it would be impossible for the meat to reach the consumer in this ideal state, but this must not prevent us from making every effort to produce the most hygienic article with the longest possible "shelf-life".

In Europe it is accepted that bacteria gain access to a normal carcass through either or both of the following:—

(i) Deep infection
(ii) Contact or surface bacterial contamination.

By "deep" infection is meant the invasion of the deep tissues, especially the musculature, that can occur irrespective of whether or not surface contamination takes place. It is believed that such bacteria arise from two possible sources and are spread via the bloodstream during the bleeding process. The first possible source is the intestinal tract which, it is believed, can infect the liver via the short portal system during the stage of agony, and this infection can enter the general circulation. The second source of "deep" infection is more obvious. This is the stab wound or throat cut made to cause the animal to bleed to death, and which again may allow bacteria to enter the circulatory system. Of the two methods of bleeding, the chest stab is the better in this regard as the trachea and oesophagus are not incised, thus aspiration blood cannot become contaminated by stomach contents. Furthermore, the speed with which the bleeding occurs will have an effect on the spread of such "deep" infection, and rapid bleeding is highly desirable. European experience is that prompt bleeding of the stunned animal in the hanging position by means of a chest stab wound is the most satisfactory means of reducing "deep" infection to a minimum.

Contact or surface contamination is the bacterial load applied to the exposed surfaces of the carcass (and the offal) during the process of dressing and subsequent handling. That such contamination must be kept to an absolute minimum is easily understood when one realises that fresh, warm meat provides an excellent culture medium for the rapid multiplication of bacteria. The same cannot be said of meat that has been coolled and meat the surface of which has already changed to a dry "skin"; this explains why it is important that carcasses be refrigerated as soon as possible after slaughter.

Contact contamination can arise from different sources and the bacteria concerned vary in species and origin. Such contaminants will fall in one or other of the following groups:—

(a) Spoilage flora — putrefactive bacteria, *Proteus, Pseudomonas,* etc. This is the flora of the environment — air, instruments, clothing, etc.
(b) Flora of the digestive tract.
(c) Flora of the hide.
(d) Pathogenic flora — conveyed by contact between diseased and non-infected carcasses.

It is understandable that most contact contamination of carcasses occurs in an indirect manner. It is not usually simply a case of direct contact between a clean carcass and one or other of the sources of bacteria mentioned above; there is usually some vehicle, such as knives, hands, etc., that transfers the infection from its source to the carcass. As it is impossible to destroy the contaminating flora, the only means of limiting the transfer of infection is by (a) limiting the amount of handling to a minimum, (b) ensuring as far as possible that only clean hands, instruments clothing, water, etc., come in contact with the carcass, and (c) ensuring that the source of infection, — e.g. hide, guts, condemned carcasses and offal, are removed from the slaughter hall as soon as possible and by a route that is clear of the slaughter line.

After studying European abattoir practice it is my opinion, bearing the above remarks in mind, that the following points, broadly outlined, constitute the main factors involved in producing carcasses of a high hygienic quality:—

(i) Rate of slaughtering and dressing of carcasses must be such that there is no crowding of the slaughter hall by slaughtermen, inspectors, carcasses, offal or equipment. In addition, the speed of operation must allow all personnel to perform their tasks properly without undue haste. It is, of course, impossible to state how fast a cattle slaughter line, for example, should operate, as there are numerous variable factors from abattoir to abattoir. Lack of space on the slaughter floor, and working at too great a pace, are considered to be the main causes of the production of unhygienic carcasses.
(ii) Objects coming constantly in direct contact with meat should be washed and disinfected as often as possible. This necessitates the easy accessibility of wash hand basins and soap, and appliances for the cleansing of

knives, cleavers, handsaws, etc. All abattoirs visited use steam for the sterilisation of such small instruments and in most cases knives etc., can be dropped into numerous strategically placed steam boxes. As is the position almost everywhere, European abattoir authorities also experience some difficulty in impressing on slaughtermen the necessity for personal cleanliness and the disinfection of small tools. Most of these abattoirs ask that workers disinfect small instruments at every break in work and also, of course, whenever contact is made with grossly infected material such as faecal matter or pus. Hooks used for hanging offal, and barrows for moving offal, are scrubbed and disinfected after the days operations are completed. In many places one sees use made of 'trail' tanks which are tanks, containing disinfecting fluid, which are positioned below the gambrel return rail and through which every returning cradle or gambrel must pass.

(iii) As far as possible, slaughtermen should be divided into those performing 'dirty' operations (e.g. bleeding, flaying) and those performing 'clean' operations (evisceration, splitting, etc.) This is, of course, part of the advantage of the "on line" system of dressing. Of great advantage too, is the division of the actual flaying operation into 'clean' and 'dirty'. The initial cut through the hide or skin contaminates the knife and if the same knife is then used to assist in freeing the hide or skin from the subcutaneous tissues the infection is transferred onto the surface of the carcass. The answer to this is for the flayer to carry two knives, one for the incising of the skin, the other for working underneath the skin.

(iv) Hides, horns, "dirty" offal, etc., should be removed from the hall as rapidly as possible and should in no way come in contact with any carcass. It is highly desirable that the overhead rail system is designed so that condemned carcasses can immediately be shunted to a separate room to await disposal.

(v) Handling of carcasses should be reduced to an absolute minimum.

(vi) It is a Danish recommendation that the dressed carcass be sprayed with water at 90°C before storing in the chiller. It is maintained that this not only reduces the surface bacterial count considerably, but also causes the surface of the carcass to dry more quickly, thus preventing much multiplication of the bacteria present on the carcass.

(vii) All carcasses should, of course, be placed under refrigeration as soon as possible after dressing is complete. Here it might be worthy of mention that virtually all the abattoir refrigeration seen in the countries visited, is of the forced-draught type.

(viii) The singeing of pig carcases is practised in all the abattoirs visited, and this is a very desirable feature. Not only does the dressed carcass have a better appearance, but the heat process undoubtedly kills a great part of the bacterial load that the carcass has contracted up to this stage in the dressing process. Hand-singeing of pigs is done in some abattoirs but most use the more efficient singeing furnace.

(ix) Most of the abattoirs seen are equipped with an "emergency" slaughter room, the purpose of which is to deal with sick and "downer" animals. Such a room lends itself to easier and more efficient disinfection, and any dangerous infection is easily limited to this one room. Furthermore, examination of a suspect carcass can be more thorough when carried out apart from the general slaughter hall.

(x) It is desirable that all persons liable to come into contact with carcasses or offal wear impervious protective clothing that is easily kept clean, such as plastic aprons.

(xi) No smoking is allowed in slaughter halls.

(xii) Floors are kept free of gross dirt during operations, and no part of the carcass is allowed to come into contact with the floor at any time. Where the "on-line" is not in use, carcasses are flayed on 'cradles' to keep them off the floor. Floors are kept clean by frequent hosing and brushing (squeeqees), and water is not allowed to be splashed onto the floor. In some instances parts of floors are washed by means of a series of water jets set into the floor, and these operate continuously. Much use is also made of grids for covering drains and gullies, the idea being that blood and gross dirt falls through the grid into the gully and the slaughtermen have a permanently clean 'floor' to work on.

(xiii) Floors and walls should be thoroughly cleaned after work each day. In the countries visited, almost every abattoir makes use of water under pressure for washing floors and walls; this is both highly effective and very quick as brushing is eliminated. To improve cleansing efficiency some abattoirs

employ steam/water mixers so that hot water is available for hosing down premises, also under pressure. At the end of each week it is routine at all abattoirs visited that the slaughtering premises be given a more thorough cleaning. The procedure is generally similar in that after an initial washdown, a disinfectant, usually hypochlorite or a halogen compound, is brushed over floors and walls using bass brooms while scrubbing brushes are used on smaller fixtures, hooks, tables, barrows, etc. Following this the disinfectant is hosed off all surfaces.

Laboratory Control of Hygiene in the Abattoir.

It is important to be able to demonstrate the weak points in hygiene in the abattoir, and when these have been remedied it is equally important to be able to show that the new measures have in fact brought about the desired improvement. When one is dealing with gross dirt, visual evidence is sufficient to indicate whether the part is clean or not. However, when interested in inapparent contamination, one is obliged to resort to the evidence of bacteriological techniques.

At all abattoirs and meat works visited in Europe, visual evidence is regarded as sufficient evidence of cleanliness when considering things such as walls, floors, protective clothing, etc. i.e. that which does not came into direct contact with meat. However, greater attention is paid to all other objects such as knives, saws, tables, hooks, etc., that must unavoidably come in contact with parts of carcasses, and it is here that they rely on bacteriological methods.

Until fairly recently the common bacterial count techniques applied to solid surfaces involved much time and effort. A common method was the so-called "ten-tube slant" technique involving ten swabs being taken of a known surface area (usually 1 sq. cm.), these being streaked individually on slants of a nutrient agar, the colony counts after incubation being recorded, graphed, a mean point selected, and finally resorting to logarithmic tables to determine the bacterial count of the predetermined area.

In 1963, L. ten Cate of Holland published details of a method he had evolved — the so-called "Agar-sausage" method (see Tijdschr. v. Diergeneeskunde 88:883). Briefly, he was able to produce a "sausage" made from an artificial casing material and containing any desired solid culture medium. In use, the end of the sausage is cut off with a sterile knife, the exposed surface is firmly pressed onto the subject under examination and a thin slice ($\frac{1}{4}$ - $\frac{1}{2}$ cm) of the agar is cut off and placed in a petri dish. In other words, an impression culture is made and sliced off, whereupon the casing is gently squeezed to expose the end of the agar for the making of the next impression, and so on. The slices are placed in petri dishes, inverted and incubated at room temperature in a cupboard or drawer for 2 - 3 days. Three or four slices will fit into the normal 10cm. petri dish. The method of making "agar sausages" is discussed later.

Use of Agar Sausages and Interpretation of Results.

No standards referring to permissible bacterial counts exist in Holland. The general feeling at present, is that it is only worth looking for Enterobacteriaceae on carcasses and equipment, as most other contamination occurring in the abattoir and meat works is of a saprophytic and ubiquitous nature. For this reasons most ten Cate agar "sausages" contain a selective medium, usually Violet Red Bile Agar, on which coliform organisms form dark red colonies. Three or more such colonies per slice is regarded as serious and warrants further investigation and the institution of remedial measures.

Professor J. H. J. van Gils has written that "in the ten Cate method we have an outstanding way of simply and objectively keeping in touch with the hygiene state of the institution." But more than this is the use of the method as a means of exerting a psychological influence on the workers in the abattoir, meat works or even in the restaurant. If workers watch the "impressions" being made on equipment for which they are responsible, and are later shown the resulting bacterial growth, this will prove a wonderful incentive to them to keep their equipment cleaner, especially if they are also shown that hygiene methods will result in fewer colonies on each slice of the agar sausage.

In Denmark the agar "sausage" method is now used exclusively to control hygiene in abattoirs and meat factories, and the ten-tube slant method has been discarded. The Danes are interested in bacterial counts, i.e. the extent of contamination present — they are not vitally concerned as to the *type* of contamination present. They feel that if too many organisms are present in meat or on equipment, this is a problem that is going to affect the keeping quality of the meat irrespective of the type of bacteria present. They further consider that by reducing bacterial counts in general, the danger of pathogens being present is also reduced. Thus, for routine work, the agar sausages contain one of the ordinary nutritive media, selective media only being used for special work.

Olgaard has published a paper setting out details of a recommended procedure when using

agar sausages, and how to express the results obtained as the number of organisms present on one square cm of surface area (see Journal of Danish Veterinary Society 49, (7): 298 - 305). According to this procedure it is necessary to take tests in series of 10 slices for each carcase or item of equipment, in addition to which 1 to 3 control slices must be provided in order to control the sterility of the "sausage" and the knife. When doing pig carcasses, Olgaard has laid down 10 sites on the carcase from which slices are made. In this way a mean count can be produced for each carcass, and it is also possible to check the counts for each of the ten sites. The 10 sites have been selected as giving the best cross-section of bacterial distribution on a carcass.

After 48-82 hours incubation, the results are recorded. Instead of registering the actual colony count per slice, only the group into which the count falls is registered, according to table 1.

able to determine at a glance which site is most highly infected. When testing equipment all point values of 5 and over are recorded in red (more than 100 colonies per slice), and when testing pork carcasses all point values of 6 and over are written in red (more than 300 colonies). This makes it easy to see any weak points at a glance.

A mean point value above 7.0 is recorded as "over 10,000". Also, if more than 5 slices show over 1000 colonies it is impossible to calculate the mean bacterial count, and again it is recorded as "over 10,000".

The Danes have set certain standards concerning utensils and certain meat products, but these are merely a guide for persons engaged in meat hygiene work. These "ideal" standards are as follows: —

(i) Bacon sides, before chilling, mean count less than 500 bacteria per sq cm, and no *site* should average more than 100 per sq cm.

TABLE 1.—GROUPING OF BACTERIAL COUNTS INTO POINT VALUE CATEGORIES.

No. of colonies	0	1–2	3–9	10–29	30–100	100–300	300–1000	Over 1000
Point value	0	1	2	3	4	5	6	8

The point value for each series of ten slices is entered on a sheet, as exemplified here for six items: —

(ii) Ditto, after chilling — counts similar.

(iii) Utensils and equipment — no slice should have more than 100 colonies and the mean count should be less than 100 bacteria per sq cm.

(iv) Raw mince, sausage, etc. — less than 5×10^6 bacteria per g.

(v) Liver paste (ready to eat) — less than 25,000 per g.

(vi) Other kinds of cooked meat products — less than 100,000 per g.

TABLE II.—EXAMPLE OF POINT VALUE SCORE SHEET FOR SIX ITEMS.

Item	I	II	III	IV	V	VI	Total	Mean	Range	Count Sq. cm.
Slice 1	6	4	5	3	4	6	28	4.7	3	700
Slice 2	6	6	4	6	4	5	31	5.2	2	1300
Slice 3	3	5	2	5	4	3	22	3.7	3	220
Slice 4	4	4	4	3	5	4	24	4.0	2	320
Slice 5	6	4	5	6	6	5	32	5.3	2	1400
Slice 6	4	4	4	4	3	3	22	3.7	1	220
Slice 7	5	3	5	3	2	4	22	3.7	3	220
Slice 8	4	3	3	3	3	2	18	3.0	2	100
Slice 9	5	4	4	4	4	5	26	4.3	1	450
Slice 10	4	4	6	5	6	5	30	5.0	2	1000
Total points	47	41	42	42	41	42				
Mean points	4.7	4.1	4.2	4.2	4.1	4.2				
Range	3	3	4	3	4	4				
Count per square cm	700	350	400	400	350	400				

Items I-VI could be individual carcasses or items of equipment. For each separate item the points are totalled vertically and converted into the bacterial count per sq cm according to the table III. Should each slice stamped on items I - VI have been taken on the same site, then the points can be totalled horizontally and converted into the bacterial count — in which case one is

TALE III.—DETERMINATION OF THE MEAN BACTERIAL COUNT PER SQ. CM FROM THE MEAN JOINT VALUE.

	0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.	3	4	4	5	5	6	6	7	8	9
1.	10	11	13	14	16	18	20	22	25	28
2.	32	35	40	45	50	55	65	70	80	90
3.	100	110	130	140	160	180	200	220	250	275
4.	320	350	400	450	500	550	650	700	800	900
5.	1000	1100	1300	1400	1600	1800	2000	2200	2500	2800
6.	3200	3500	4000	4500	5000	5600	6300	7100	7900	8900
7.	10000									

It is very important to remember that the bacterial count determined by means of agar sausages is not an absolute count. The number of bacteria or microcolonies transferred to the agar will depend on the smoothness of the surface, the presence of moisture, and perhaps on other factors. Counts made in this way are therefore relative, and figures obtained from two different types of surface cannot be compared.

PREPARATION OF AGAR SAUSAGES.

The casing used in Holland and Denmark for the preparation of agar-sausages is a polyamide (Rilsan) artificial casing (calibre 50/004) that is able to withstand a temperature of 140°C.*

The casing is cut into lengths of 65 cm, each of which is securely tied at one end with good quality string. If several media are used different coloured string can be used to identify each medium. 300 ml of molten agar medium is poured into each length of casing, each of which is then sealed by tying off the open end. The casing is by no means full. The sausage is then hung over a frame so that approximately half the medium is at each end, and a small hole is cut in the middle of the casing. This is to permit free air to escape from the sausage during heating without bursting the casing. The frame is then placed in the autoclave and sterilisation is carried out in the usual way. As soon as the autoclave is opened each casing is tied off on either side of the hole to prevent organisms entering the sausage. Allow the sausages to cool somewhat, because the casing does not shrink with the agar, but before the agar solidifies the casing is twisted so that it tightens on the agar and becomes very firm. The final tying of the sausage is made close up against the agar to prevent the casing untwisting.

In this way twin sausages, each about 17 cm long, are made together in the original length of casing of 65 cm. The diameter of the sausage is about 34 mm and the surface area of a cross-section is about nine square centimetres. The sausage will keep for an unlimited time as the agar is unable to dry out.

C. *PROCESSING AND CONTROL OF ABATTOIR BY PRODUCTS.*

1. HOLLAND. In the Netherlands there are three very large destructors (as dry-rendering plants are known) and some 9 or 10 smaller plants. Almost all are co-operatively owned and strategically situated in rural areas. They are responsible for the collection of all condemned material and blood from abattoirs and all dead animals within their area of operation.

Two of the larger destructors were visted viz. at Son in North Brabant and at Bergum in Friesland. These have an annual intake of raw material of 75,000 to 80,000 tons each and work a 24 hour day for six days per week. Each carries a staff of some 165 workers made up approximately as follows:— 10 laboratory technicians, 20 administrative workers, 35 drivers and 100 engineers, plant operators, etc. Steam capacity of both plants is in the region of 30 tons per hour, and is produced by oil-fired burners. Both plants produce carcass meal, blood meal, tallow, bone meal and feather meal and the Bergum plant in addition produces mixtures to clients' requirements.

The basic principle safeguarding the sterility of the end-product of the plant is the absolute separation of untreated material from the area where the sterilised products are handled, and this includes the labour force working on either the 'dirty' or 'clean' sides. Movement of raw, partially treated and final products is done throughout by means of screw conveyors, which makes for cleanliness and ease of handling. Occasionally a check on sterilisation is made by including a 'Spore Strip', containing heat-resistant spores of *B. stearothermophilus* in a digester with a load of raw material — when cooking is complete the strip is suspended in broth and incubated; no

*Rilsan casing is available from S.A. Organico, 23 Avenue F. D. Rooseveldt, Paris VIII.

growth indicates that heat treatment has been adequate. Digestion is carried out in the same manner as in this country, and according to law the temperature must exceed 100°C for a minimum of 3 hours or 130°C for more than 20 minutes. It is interesting to note, however, that the Dutch digestors have the steam jacket extending over both ends of the machine. This makes for more efficient and quicker cooking. Temperature is automatically controlled and is recorded by thermographs.

Fat extraction. At the Bergum plant fat extraction is carried out using a solvent (heptane) and the plant is regulated so that the carcass meal has a final fat content of not less than 4%. The main advantage of the solvent extraction method is that the maximum quantity of fat can be removed from the meal, and fat is, of course, the most valuable product of the plant. However, the disadvantages of this method are (i) the very high capital cost of the plant, (ii) the element of danger that is ever present when using highly inflammable solvents, and (iii) the meal can be quite dusty if too much fat is removed. The people concerned are adamant that meal produced in this way is as palatable to livestock as that produced by any other method.

At Son, fat is removed from the digested material by means of 'expellers', which are in fact automatic presses which squeeze the tallow from the meal. This plant has used this method for four years and the management is entirely satisfied. The price of one such 'expeller', in Holland, is the equivalent of approximately R15,000 and the maximum capacity of a single machine is 900 Kg of non-defatted material per hour. The final fat content of the meal produced is 10 to 12%, but if required the expeller can reduce this to 8 or 9%.

Fat clarification. This process is carried out at Bergum using the same method commonly used in this country viz. the dirty tallow is pumped into conical settling tanks where it is boiled with water, allowed to settle and the water decanted; the fat is then steamheated and centrifuged in a fat clarifier.

A continuous decanter, followed be centrifugation, is used at Son. The continuous decanter consists basically of an internal screw, which carries the fat, and which revolves at a slightly lower speed than the jacket surrounding it; thus the fat is moved forwards while being spun at high speed, so removing any solid particles which are drained off and returned to the expeller.

Blood drying.

No plants in Holland coagulate blood before it is put in a digester. This means, of course, that a longer cooking time is required (approximately 8 hours) but this is offset by the saving of the 'bloodwater' which contains about 2% protein. Possibly sterilisation is better in this way as no big coagula go into the digestors. However, at Bergum the blood is only cooked for two hours after which time the moisture content is reduced to about 50%; it is then passed through a hot-air tunnel for further drying so that the meal has a final moisture content of 8 to 10%. It is averred that this method is cheaper than long cooking and that the quality of the meal is better as there is less burning of protein against the steam jacket of the digestor. The same digestors are used repeatedly for blood, and about 100 lb of old tin cans are put in with each load to scrape the walls of the digestor clean.

Odour control.

A destruction plant, by the very nature of the service performed, produces unpleasant smelling gases, and it is important that as little of these gases as possible be discharged into the atmosphere. This is especially true where a by-products plant is situated in a built-up area. The usual procedure in Holland is to wash vapours from digestors in ejector condensers, but this is not the complete answer as certain of the more objectionable vapours are insoluble in water. It is possible to burn these insoluble gases, but the great drawback to this procedure is that they have a marked corrosive effect on pipes, boilers, etc. However, a very efficient system of dealing with this problem was seen at the Bergum plant. Vapours from all digestors are piped to a central point where they are waterwashed under vacuum — so-called "barometric" condensers — after which the non-condensable gasses are sucked off by vacuum and are treated with a citronella oil/water mixture in a "homemade" plant. Citronella oil costs about R0.80 per kg and the Bergum plant used two to three kg per day.

2. *Denmark.* In Denmark, as in Holland, co-operative dry-rendering plants process all abattoir waste and are also responsible for transporting and processing all dead animals. One plant only was visited, namely that at Ortved, which processes material from the whole island of Zealand. Throughput is in the region of 50,000 tons of raw material per annum of which 80% is derived from abattoirs. Only blood meal, carcass meal and tallow are produced.

The processes used at this plant are the same as encountered in Holland with the exception of the method used in the manufacture of blood meal.

The method of drying blood is most interesting and is carried out automatically. The raw blood

is preheated to 50°C while being agitated and is then passed through a heater which rapidly raises the temperature to 90°C, causing coagulation; a so-called "de-sludging" machine then separates off most of the water and delivers the blood with a moisture content of only 50%. This is then transferred to a digestor where a "cooking" time of only 1½ hours is required to bring the moisture content down to 10%. The 'desludger' can handle 2¼ tons of raw blood per hour and the cost of such a machine is approximately R9,000. Alfa Laval are the manufacturers of the machine.

Danish regulations state that sterilisation of raw materials must be done in a dry renderer in which the contents are heated under constant stirring to at least 125°C for a minimum of 15 minutes. There is very strict separation of "clean" and "unclean" departments of the plant. There is no routine examination of specimens to ensure that sterilisation is adequate or to determine whether or not recontamination of the end-product has occured, but all dry-rendering establishments fall under the control of a Supervising Veterinary Officer whose duty it is to ensure that there is no contact between "clean" and "unclean" sections, and that sterilisation temperatures used are as prescribed in the regulations.

3. ENGLAND. No abattoirs process their own condemned and waste materials. Knackers contract for the material which they remove to their premises daily. There are numerous knackeries scattered throughout the country and mostly they are very small plants. Virtually no control is exercised over these establishments, but the authorities seem to feel that no control is necessary. Condemned meat from abattoirs is stained with a green dye to prevent sale as fresh meat. Knackeries also collect dead animals from farms (although not obliged to do so) and farmers are not obliged to have their dead livestock destroyed by a knackery. Dead animals are often prepared at the knackery for sale as pet food.

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I wish to express my sincere appreciation to:

1. The S.A. Livestock and Meat Industries Control Board for the award of the Fellowship which enabled me to undertake this study tour;
2. The Director of the Municipal abattoir and the Corporation of the City of Durban for permission to proceed on this study;
3. The numerous persons at universities, abattoirs and other institutions in Holland, Denmark and England who made it possible for me to gain first hand knowledge of their systems and methods.

BOOK NEWS

In the December 1966 issue of this journal we announced the Modern Veterinary Reference Series now being issued by American Veterinary Publications and giving a comprehensive review of current progress in veterinary medicine.

To the first three volumes then listed, namely, Progress in Equine Practice, Progress in Swine Practice and Progress in Feline Practice we can now add two more, namely:

PROGRESS IN CANINE PRACTICE PART I (Surgery, anaesthesiology and radiology), and PROGRESS IN CANINE PRACTICE PART II (infections, infestations and neoplasms).

Further, it is anticipated that the series will be complete by the end of the year by the publication of the third portion of Canine Practice (non-infectious diseases, reproduction and nutrition), the three volumes on Cattle and Sheep Practice, and finally Progress in Animal Hospitals.

Judging by the demand this series of 10 volumes is proving to be very popular, and we are now taking orders, not only for those volumes that are already available but also for those still in production. Orders placed in advance will receive priority when the first consignment of each volume is received. The price is R12.75 per volume.

In the running of a private veterinary practice the business side is almost as important as the professional aspect and largely determines the margin of profit. Yet it is something that receives no or very little attention in the curriculum of training institutes. We however have good news for small animal practitioners by announcing the publication of a guide book which is certain to be of great assistance in conducting a practice more efficiently and economically. This is THE BUSINESS MANAGEMENT OF A SMALL ANIMAL PRACTICE, by Harvey Saner. It covers a wide field such as accounting and bookkeeping, ethics, legal responsibilities, the expert witness, efficiency and economy in the lay-out of a hospital, purchasing of a practice and goodwill, insurance, and auxillary personnel. 248 p.p. R7.15.

Beef production is at present undergoing swift and radical changes, and even in South Africa the days when beef cattle were turned out into the veld and only brought in when the owner thought they were ready for slaughter are coming to an end. Beef production is fast becoming as scientific an industry as milk production. A new publication entitled PLANNED BEEF PRODUCTION, by S. Williams & C. D. Edgar provides farmers and others concerned in beef production with valuable information on the latest developments and methods. Concisely written (189 pages) and with 80 illustrations it deals with subjects like the kind of beef to produce, rearing the calf, the grazing concept, inside feeding of beef cattle, their health and habits, the business aspect, and the end product. R3.75.

We are pleased to announce the publication of the *Third Edition* of the famous and popular MERCK VETERINARY MANUAL, the most important single- volume source of a wide range of information for the veterinarian. In addition to more than 300 chapters of concise authoritative coverage of animal diseases the new book includes seven other major parts:

TOXICOLOGY, POULTRY, FUR LABORATORY AND ZOO ANIMALS, NUTRITION, ADDENDUM REFERENCE MATERIALS, PRESCRIPTIONS, INDEX.

There are 25 new chapters, and the eight major parts are divided into 20 principal sections, each thumb-indexed. It is richly bound in waterproof, vermin-resistant, gold-stamped Sturdite. R9.75.

The fifth edition of MONNIG'S VETERINARY HELMINTHOLOGY AND ENTOMOLOGY by Lapage is now out of print. A new edition under the title HELMINTHS, ARTHROPODS AND PROTOZOA OF DOMESTIC ANIMALS by Soulsby is scheduled for publication early in 1968.

NOTES ON THE SPERMATOZOAL MORPHOLOGY OF SOME UNGULATES.

J. C. MORGENTHAL*.

SUMMARY

Smears of epididymal sperm of the hippopotamus, kudu, impala, blesbok, red hartebeest and blue wildebeest were examined and measured microscopically. Distinct differences in both size and shape between the spermatozoa of the six mammals were found.

INTRODUCTION

The study of reproduction in game animals has progressed considerably in recent years[1]. Aspects investigated include the anatomy and histology of the organs of reproduction, the physiology of reproduction, sexual behaviour, breeding habits and gestation. Most work has been devoted to the female of the species. Although the marsupial sperm has received considerable attention[2] relatively little information on that of ungulates is available. The present study was to investigate some morphological aspects of the sperm of the hippopotamus, *Hippopotamus amphibius;* red hartebeest, *Alcelaphus buselaphus;* blue wildebeeste, *Connochaetes (Gorgon) taurinus;* impala, *Aepyceros melampus;* kudu, *Tragelaphus (Strepsiceros) strepsiceros* and blesbok, *Damaliscus dorcas phillipsi.*

PROCEDURE

Semen samples were collected from hippopotamus, wildebeest, impala and kudu shot in the Kruger National Park; blesbok from Harrismith, O.F.S., and hartebeest from the Kalahari Gemsbok Park.

In the case of the hippopotamus the sperm were collected one hour after death, and from the other species within 30 minutes.

The semen was collected from the *cauda epididymidis* as described by Lasley and Bogart[3], but saline was used instead of buffer solution. A drop of semen of each animal, except blesbok, was mixed with two drops of a 10 per cent solution of nigrosin, and the semen smear made according to Rao and Hart[4]. The blesbok semen smears were stained according to Karras' method[5].

The dimensions of the sperm of each species were obtained by measuring the total length, length of the middle piece and neck, length of the head and width of the head of 50 different cells by means of an ocular micrometer[6], at a magnification of 1000.

RESULTS AND DISCUSSION

The most characteristic, and also the smallest sperm is that of the hippopotamus. The sperm are conspicuous because of a constriction of the head approximately 1.3 microns from the posterior extremity. The spermatozoa of the hippopotamus are furthermore, not comparable with those of the taxonomically related domestic pig in which the sperm are not only longer and bigger but also lack the prominently constricted head.

The heads of the spermatozoa of kudu and impala were found to be both longer and broader than those of the rest of the wild ungulates studied. The main difference between kudu and impala sperm is that the head of kudu sperm resembles that of the bovine in shape, while that of impala is similar to ovine sperm.

The total lengths of kudu and impala spermatozoa are approximately the same, but the middle piece of impala sperm is shorter than that of the kudu, although the heads of the impala sperm are both longer and broader.

The blue wildebeest, red hartebeest and blesbok are the three species of which the sperm heads are larger than those of the hippopotamus, but smaller than the kudu and impala spermatozoa. Of these three species the heads of the wildebeest sperm are the largest, with the red hartebeest and blesbok following in decreasing order. In shape the sperm heads of the three species mentioned all resemble ovine spermatozoa, with certain exceptions. The spermatozoal head of the blue wildebeest is more rounded laterally than is sheep's sperm; the heads of hartebeest spermatozoa are slightly flattened laterally, and the anterior extremity of the heads of blesbok sperm have a blunter appearance when compared with ovine sperm heads.

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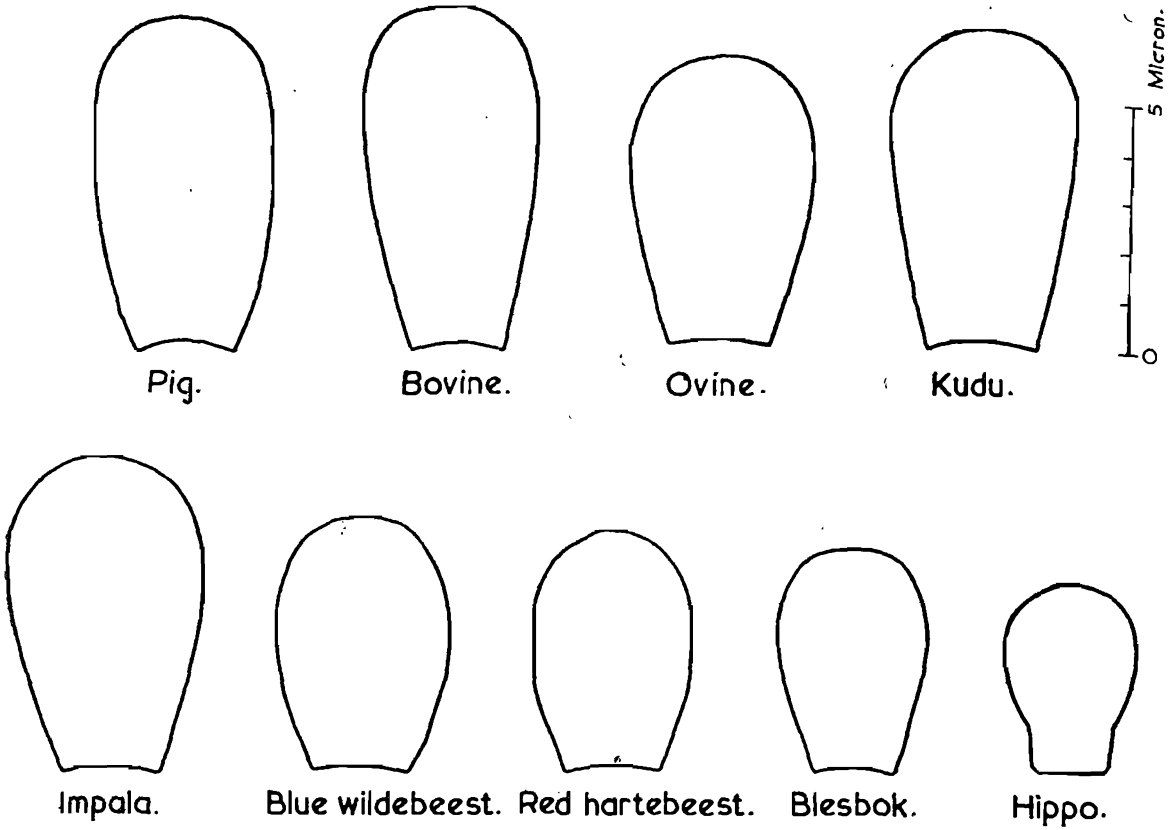

Fig. 1 — Drawings of the spermatozoal heads of three domestic and six wild animals, demonstrating the variation in size and shape.

TABLE 1.—MEANS AND STANDARD DEVIATIONS OF SOME MEASUREMENTS (IN MICRONS) OF THE SPERMATOZOA OF THREE DOMESTIC AND SIX WILD ANIMALS.

Species	Length of Head (mean ± S.D.	Width of Head (*maen* ± S.D.)	Length of Middle *piece* (mean ± S.D.)	Total Length (mean ± S.D.)
Pig	6.55 ± 0.01	3.47 ± 0.02	11.88 ± 0.03	39.96 ± 0.1
Ovine	5.78 ± 0.01	3.54 ± 0.02	10.27 ± 0.02	45.69 ± 0.02
Bovine	6.77 ± 0.01	4.27 ± 0.01	9.83 ± 0.01	53.53 ± 0.1
Hippo	3.77 ± 0.02	2.65 ± 0.02	7.85 ± 0.01	33.49 ± 0.1
Red Hartebeest	4.82 ± 0.01	3.06 ± 0.01	7.23 ± 0.02	48.37 ± 0.2
Blue Wildebeest	5.01 ± 0.03	3.56 ± 0.02	7.86 ± 0.02	46.77 ± 0.3
Blesbok	4.53 ± 0.02	2.97 ± 0.02	7.7 ± 0.03	50.82 ± 0.3
Kudu	6.41 ± 0.02	3.75 ± 0.02	8.99 ± 0.02	45.54 ± 0.3
Impala	6.52 ± 0.02	4.13 ± 0.02	7.63 ± 0.02	46.15 ± 0.2

The total length of blesbok sperm is considerably greater than that of either the red hartebeest or the blue wildebeest, although the other dimensions are smaller.

The semen of all the animals studied contained various types of abnormal sperm. Cytoplasmic droplets were present in all the smears, and the majority of these droplets were situated on the lower part of the middle piece, or upper regions of the tail. Curled tails were abundant in the semen of all six animals, particularly in the hippopotamus and kudu. It must, however, be borne in mind that the semen was collected from the epididymis and should be considered as being immature, as manifested by the presence of these aberrations[3].

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BOOK REVIEW

GENETICS OF THE DOG. (The basis of successful breeding). Marca Burns and Margaret N. Fraser. (1966) Olivert, Boyd, Edinburg. Pp. 230, Plates 19, Figs. 3. Published Price 45 shillings.

This book is a new edition of a work first published by the Commonwealth Agricultural Bureau in 1952. Recognition of the importance of inherited defects in dogs, and the advances in research on canine behaviour have made a revision of the original edition necessary.

The list of references (20 pages long) is proof of the welter of detail incorporated in the book. It is inevitable that there should be contradictory opinions among these sources, and several inaccuracies are presented as facts.

The statement made with regard to retinal atrophy ". . . it is a safe rule never to breed from an animal showing an abnormality" is the basic theme of the book, but the authors have in several instances, plotted a programme to breed out existing defects without sacrificing the desirable points of the breed.

" . . . the genetical data are very unsatisfactory . . ." is a frequent comment on the reports referred to. This must have been a source of frustration to the authors, and is also the flaw in this book. Many of these reports show careless observation and illogical, speculative conclusions.

The section on behaviour has been prepared by Fraser. The new work in this field was performed under laboratory conditions by such workers as Fox and Pfaffenberger. The reviewer regards this as the most enjoyable part of the book.

A mass of usable facts was gleaned from this book, in spite of the genetical reasonings on some points being quite mystifying to me. The majority of dog breeders that we know will find the book incomprehensible, but those who have a grasp of the basic facts of inheritance may be urged to read this work. Veterinarians, particularly practitioners, will find solutions to, or explanations for, many problems that crop up in their daily dealings with dogs and breeders.

P. H. le R.

SURGERY OF BOVINE IMPOTENTIA COEUNDI.

I. Introduction: methods of examination; analgesia and anaesthesia; general treatment.

C. F. B. HOFMEYR*.

SUMMARY

Details are given of general routine methods of examination, analgesia and anaesthesia, and of general treatment, based on observations on a series of 176 bovine cases of *impotentia coeundi* due to pathology of the penis and adnexa.

The significance of the condition, particularly in the light of aetiological factors operating in South Africa, is evaluated.

INTRODUCTION

This is the first of a succession of papers based upon observations over a period of eight years on a series of 176 cases of surgical *impotentia coeundi* in bulls, upon experiences gained by application of various forms of therapy and upon the final conclusions drawn. Details have been presented previously[1]. This condensed version is aimed mainly at being of use to surgeons in particular and practitioners in general.

Sterility in the bull may be due to absence of *libido*, to *impotentia generandi* (as result of semen deficiencies) or to *impotentia coeundi*.

Impotentia coeundi embraces all pathological or pathological physiological states which render the bull physically incapable of service whether affecting the penis and adnexa, or located elsewhere in the body. The latter category includes pathological conditions of the spine[2] and hindquarters[3], painful arthritis[4], particularly tarsitis, and panaritium of the hindfeet; these are either so painful as to discourage mounting, or may prevent it by mechanical interference or by causing weakness of the hindquarters. These conditions may be associated incidentally with surgical pathology of the penis or adnexa.

The surgical conditions of the penis and adnexa form the main concern of this series of papers. In all instances there is mechanical interference with the penis or adnexa so as to prevent intromission at attempted service. A few odd types of cases have been described in the literature but not encountered in the series of cases observed, e.g. failure of the sigmoid to straighten[5].

To achieve some system in presentation, the surgical pathology of the penis and adnexa is classified primarily on an anatomical basis. Besides creating a definite order, anatomical rather than aetiological considerations determine the therapeutic approach. (The pathology of different parts of the penile apparatus, although often identical in cause and result, commonly requires widely different treatments.) Conditions of a particular anatomical region are then subdivided according to the underlying pathology of each, as follow:—

A. Short, contracted or immobilised *Musculi retractores penis.*

B. Fibrous adhesions to the distal bend of the sigmoid flexure.

C. Penile and peripenile haematoma, abscessation and adhesions in the region between scrotum and *fornix praeputii.*

D. Stenosis of the preputial mucous membrane, excluding the *orificium praeputiale.*

E. Surgical conditions of the preputial skin and prolapse of the parietal mucous membrane.

F. Surgical conditions of the *glans penis.*
   (a) Bleeding haemangiomata.
   (b) Neoplasms.
   (c) Abscesses and wounds.
   (d) Paraphimosis.
   (e) Phallocampsis (deviated penis).

G. Miscellaneous developmental defects.

Since there are major differences between man and bull concerning the anatomy of the penis and thus also the pathological — anatomical conditions encountered, a new terminology had to be introduced and is set out briefly below.

1. "*Retentio penis*": inability of the penis to be protruded, irrespective of cause.
   (a) "Cohibition" of the penis: retention because of restraining mechanisms proximal to the glans.

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(b) "Phimosis": retention of the penis as result of stenosis or narrowness of the preputial orifice.

2. "*Protentio penis*": protrusion of the non-erected penis, irrespective of cause.
   (a) Paraphimosis: Failure of the protruded glans penis to return to its resting state inside the prepuce due to constriction of the latter.
   (b) Prolapse of the penis due to failure of the retracting mechanism.

These terms will henceforth be used within the limits of their definitions as given. Where, in the literature reviews, various authors are quoted using "phimosis" and "paraphimosis" in a wider sense than used here, such terms will be placed in quotation marks.

Incidence and significance

It is quite clear from a perusal of the literature, that, although surgical pathology of the bull's penis is very important and the incidence is fairly high[6,7,8], little has been published in this field[8-10]. The small number of publications shrinks even more when those dealing with the clinical and therapeutic aspects only are taken into account. In this reduced group a number of reports deals with one or a few cases only, while it is difficult to assess the value of therapy in some others. Comparatively little new knowledge in this field has appeared in print for decades.

In South Africa most cattle breeders have suffered grievous economic losses from *impotentia coeundi* due to pathology of the penis and adnexa: valuable genetic material has been lost. It is possible that it is more common in South Africa than in most other countries for a variety of reasons.

Many cattle farms operate on an extensive basis. Free service, and not hand service, is the usual practice. These factors militate against prevention of service injuries that could be preventable by closer supervision. Injury, once sustained, may not be detected for a long time; mild injuries may thus be aggravated by further injury, or by lack of early attention until the condition progresses to a much more serious phase. As totally unskilled, frequently negligent labour is commonly employed, abnormality of the penis may not be detected, or not be reported if seen. In the cases encountered[1], certain bulls had been kept in the herd for some years before the owner discovered that they were incapable of service.

In almost all cattle areas tick life abounds and is very active, unless regular dipping is carried out. Particularly the species of the genera *Amblyomma* and *Hyalomma,* which have long mouth parts, favour the ventral abdomen, axilla and inguinal region. Besides inflicting direct local injury, the tick bites provide access for corynebacteria which are very prevalent, thus producing abscesses of the external genitals, or bacteraemia and resultant infection of the haematoma, formed after bruising of the penis.

Certain environmental features, common in most of the extensive cattle breeding areas, are a distinct hazard in terms of trauma to penis and adnexa. These consist of coarse vegetation, such as grasses with sharp awns, scrub bush and thorn trees, barbed wire fencing and roughness of terrain. It is not uncommon for a bull to lacerate penis or prepuce if attracted by a cow in an adjoining camp, or to lose his footing whilst mounting a cow.

Some conditions of the penis and adnexa are apparently of developmental origin. This focuses attention on their heritability. Whether treatment of cases, where genetic transmission may possibly occur, is justifiable or not, is a matter of extreme complexity. As no perfect cattle exist, every fertile mating inevitably transmits some undesirable characters. Where inheritance of certain characters has been established beyond doubt, it still remains to balance the desirable characters against the undesirable ones, before a decision concerning elimination of the animal from breeding can be reached. In abnormalities of the penis, however, the issue is further clouded by the fact that some conditions are stated to be of genetic origin, without submission of sufficient factual evidence. In individual cases exhaustive investigation into the progenitors and progeny of the propositus may be required; even then, care should be taken to eliminate environmental factors which may be responsible for the abnormality.

In the Netherlands, legislation was passed years ago outlawing treatment for contracted (short?) retractor penis muscles, because of its heritability. Even in this respect arguments will be presented which raise the question whether the condition does not include two distinct abnormalities. If this is so, the problem then arises whether *both* are heritable, whether they are genetically linked or separate and whether their transmission is identical or not. Whatever the position may be regarding inheritance in certain penis abnormalities, no objection can be raised to treatment where the whole calf crop is destined to be sold as beef.

As no legislation exists in South Africa preventing curative treatment of any penis abnormalities, all cases of *impotentia coeundi* should be considered for possible treatment. If any hereditary cause is suspected the owner should be informed accordingly and treatment should be withheld at his request.

Routine methods of examination

The description here will refer first to the general examination and then to the special examination of different parts of the penis and adnexa, more or less in the order in which the pathology of these parts is dealt with in these articles.

A general clinical examination must be conducted to determine whether the bull is in normal health. As disease of the nerves may account for failure to serve, attention must be paid to the spine; integrity of the spinal cord and of the peripheral nerves, particularly of the lumbo-sacral plexus, must be established. The joints of the limbs and the condition of the feet have to be examined.

Visual inspection of the external genitalia is carried out and followed by palpation to detect obvious deviations from normal. At this stage special methods of physical or chemical restraint generally are not required.

If no obvious cause of *impotentia coeundi* is revealed, the bull must be encouraged to mount a test cow specially kept for the purpose and brought into oestrus by injection of appropriate hormone, or any cow in oestrus, so that the mechanical function of the penis may be observed. Artificial oestrus in the cow appears to cause a reluctance on the part of some bulls to jump her. Others, particularly Afrikaner and Brahman bulls, are frequently shy of the presence of human beings, so that this aspect of examination sometimes extends over a few weeks. Various subterfuges may have to be used eg. keeping a special watch to observe spontaneous erection during early morning.

Further examination sometimes requires the use of a tranquillizer and/or pudendal nerve block. When such is the case, preparation is made for operation, if necessary, so as to avoid repeating the transquilization and/or analgesia for surgery later on.

If there are any signs of partial or complete cohibition, pudendal block must be employed as routine measure. If the tip of the glans can be brought into view, it is grasped with a gauze swab and withdrawn as far as possible. If withdrawal is impossible in this manner, the penis may be brought into view by inserting whelping forceps into the preputial cavity and grasping the tip of the glans behind the galea and then catching it with gauze. If this manoeuvre fails on the standing bull, he must be cast and the four feet tied together. This will assist delivery of the penis. Any pathology in the preputial cavity must then be excluded by visual inspection if the penis can be brought out far enough, or, if not, the cavity must be explored with the gloved, lubricated forefinger.

To exclude adhesions around the penis in the area between the *fornix praeputii* and the scrotum, the tissues surrounding the penis are carefully palpated through the skin to identify a mass or bands of scar tissue. If any are found, the scar tissue is moved back and forth to determine if the penis is involved. In addition, an assistant should pull the glans intermittently to establish whether the movement is transmitted to the granulation tissue. In this manner the significance of granulation tissues may be determined with certainty.

The same methods are effective to exclude adhesions in the region of the sigmoid flexure behind the scrotum, although interpretation of findings is more difficult because the penis lies more deeply under the skin.

Finally, the state of the *Mm. retractores penis* and adnexa must be assessed. If pudendal block has been effective, these muscles should be flaccid. Proof of the efficacy of pudendal block is provided by the complete anaesthesia of the glans and preputial mucous membrane. If the *Mm. retractores penis* are short or contracted, the muscles can be felt like cords behind the scrotum. Finally, to confirm pathology of these structures, the surgeon palpates them while an assistant intermittently pulls on the penis. These muscles then undergo various states of tension in direct relation to that applied by the assistant.

If the examination reveals fibrous tissue strands or a mass of granulation which limits the excursions of the penis, more information has to be gathered by careful local examination in the following manner: while the penis is being pulled forward, the site of the adhesions is determined visually and by palpation. If the skin over the lesion moves with the movement of the penis, these two structures must be considered directly adherent. If the penis moves a short distance before skin tension is observed, a layer of fairly normal fascia surrounds the penis. Should the skin over the lesion be slightly movable before the mass limits the movement, fairly normal fascia lies under the skin and the mass is thus fairly free between skin and penis; if not, skin and scar tissue are firmly joined together. Unfortunately, it is not possible to identify each of the triply telescoping layers of connective tissue surrounding the penis with exactness on clinical examination, although a tentative opinion may often be ventured regarding which layer is affected. The results of this examination have a bearing on prognosis, as will be discussed later.

If any pathology is found, or suspected to exist, distal to the *fornix praeputii,* two special examination techniques may be undertaken to determine the size of the preputial cavity and the presence of any narrowing. They form part of the general examination procedure whenever considered necessary, but need not be applied as a routine measure. The first method is one of inflation. A thin, blunt-pointed, metal tube, like a teat siphon, is connected to an oxygen cylinder by means of plastic tubing and inserted into the preputial orifice. A rubbershod bowel clamp is placed over the end of the preputial skin to effect an airtight closure around the metal tube. The cylinder cock is opened slightly so that oxygen is blown into the preputial cavity until it is fully inflated. Palpation of the ballooned cavity through the skin greatly facilitates detection of irregularities in preputial contour (see plate 1).

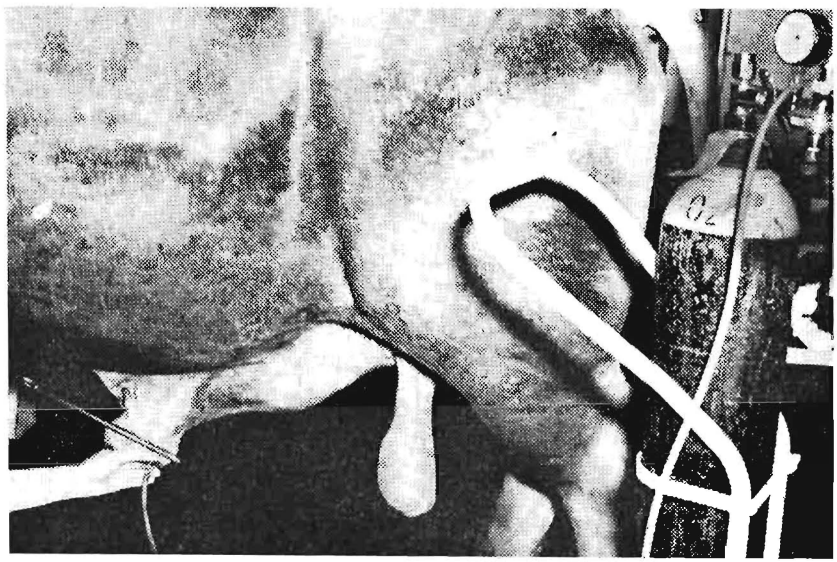

Plate 1. — Prepuce inflated with oxygen to reveal stenosis.

The other technique is simply one of visual inspection. If ordinary manipulation fails to allow grasping the *galea glandis,* the pudendal nerve must be blocked. Tissue forceps are inserted into the ostium of the prepuce and the parietal mucosa grasped and drawn outwards. A second pair of forceps is inserted more deeply at a different point of the circumference, the mucosa grasped and drawn out. This manoeuvre is repeated, using a third pair of forceps and even a fourth pair, hand over hand, until the glans can be grasped or the stenosis brought to the surface. In this manner a great deal of information may be obtained.

Observation of erection will show whether the penis is straight or not. The glans and the parietal mucous membrane are inspected for any deviations from normal, including scars or congenital abnormalities.

Examination of the preputial orifice or of prolapsed prepuce or of *protentio penis* does not require pudendal block and can always be conducted satisfactorily if the bull is kept under proper control. Sometimes very fierce or restive bulls require ataractic premedication.

Routine Methods of Analgesia and Anaesthesia.

For tranquillization, chlorpormazine (Largactil, Maybaker) is injected intramuscularly at 1-2 mg/kg bodyweight in fractious bulls to permit examination or application of a local analgesic. Alternatively, acetylpromazine (Boots) may be used, at the rate of 0.5 - 0.1 mg/kg intramuscularly. For this purpose the effects of the two drugs are about the same, but the local reaction of the latter is insignificant as compared to that of the former.

The value of tranquillizers is well established; the results obtained by Lundvall and Campbell[11] with chlorpromazine are representative. These drugs, besides inducing tranquillization, cause more or less relaxation of the *Mm. retractores penis,* depending on the dose. The intramuscular route is always employed one half to one hour before examination or operation. Intravenous in-

jection is not favoured, as it sometimes causes fatal collapse in cattle.

To induce local analgesia, satisfactory results may be obtained with two per cent w/v solution of either procaine hydrochloride (Procaine hydrochloride, Maybaker, and Novocaine, Winthrop) or lignocaine hydrochloride (Versicaine, Maybaker; Xylotox, Saphar Laboratories, or Xylocaine, Duncan Flockhart). The latter is preferable because of more rapidly induced analgesia. Where injections into dense fibrous tissues have to be made, it is combined with hyaluronidase to facilitate dispersion. For local infiltration to permit operation, as much analgesic is injected as required: 10-20 ml a side for pudendal nerve block and 8-12 ml for epidural analgesia. The latter injection is always made into the first intercoccygeal space for the sole purpose of operating in the perineal area. Although this approach and a very high dosage of analgesic to produce prolapse of the penis was standard practice some years ago, this method is not recommended. Besides interfering with locomotion, it is known that injection of some or more of analgesic occasionally causes paraplegia, paresis or permanent paralysis of the tail.

Pudendal nerve block plays an important role in the examination and in the treatment of the types of cases to be described. It causes relaxation of the *Mm. retractores penis* and analgesia of the penis and preputial mucous membrane. If the penis prolapses spontaneously, it can be inspected. If a hindrance prevents prolapse, the application of instruments to the penis or mucous membrane permits visual examination of the parts brought into view by traction. Pudendal block also allows painless surgery on the penis and preputial mucous membrane. Larson[12] introduced pudendal nerve block to effect relaxation of the *Mm. retractores penis*. He investigated the ischio-rectal and the lateral approaches and discarded the latter in favour of the former. Achmetli[13] investigated the ischio-rectal approach, while McFarlane[14] re-introduced the lateral approach. I have employed both methods with good results. There can be no doubt at all that pudendal nerve block has superseded other methods of extruding the penis for examination and surgery. Analgesia of the penis and preputial mucous membrane occurs in 5-15 minutes. It may persist for as long as two hours. Unless a hindrance is present, the penis prolapses from the sheath for the duration of the analgesia.

Where possible or convenient, the above form of analgesia is employed for operation. The bull is usually operated upon standing or, if indicated, in lateral recumbency. Where the latter contingency is anticipated or if general anaesthesia is to be employed, feed and water are withheld for 24 hours preceding operation to minimise the risk of bloat and regurgitation of ruminal contents. General anaesthesia is induced with intravenous chloral hydrate, 5 g per 50 kg bodyweight, in 10 per cent w/v aqueous solution to which is added 30 mg atropine sulphate to control salivation. This is supplemented by pentobarbital sodium (Sagatal, Maybaker) intravenously as needed, or occasionally, by local infiltration analgesia. As alternative, anaesthesia is induced by intravenous injection of one g thiobarbiturate (Intraval sodium, Maybaker, or Pentothal sodium, Abbott) per 90 kg bodyweight followed by halothane, (Fluothane I.C.I.) inhalation anaesthesia.

General Treatment

All operations must be performed under as strict aseptic conditions as conditions permit. Where asepsis is in doubt or the operation area is infected, 3-5 mega units penicillin is injected intramuscularly daily for 3-6 days. When an abscess has been opened or the preputial mucous membrane and penis have been operated upon, flushing of the abscess or preputial cavity should be carried out daily with 1:500 acriflavine in glycerine (henceforth briefly referred to as A/G). In all cases hot water irrigation or infrared irradiation is applied daily for 10-20 minutes.

When adhesions are present, or surgery has involved the preputial mucous membrane, massage is an important treatment. This is usually delayed for 8-10 days postoperatively in case of open surgery, in order to allow the divided tissues to unite. The technique consists of gripping the skin over the area involved and moving it firmly but gently back and forth, tensing, without excessively straining, the underlying tissues. This is carried out daily for 10-20 minutes. The routine of A/G flushing and hot water irrigation have been applied to the majority of cases. The use of other drugs or treatments will be indicated at the appropriate places.

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DEMODICOSIS IN SHEEP IN SOUTH AFRICA.

R. DU TOIT* and O. G. H. FIEDLER**.

SUMMARY

Ovine demodicosis (*Demodex ovis* Raillet (1895) infestation) in South Africa is recorded for the first time. Only one case has so far been found. The literature on the condition is briefly reviewed and the case described. Attempts at transmission were unsuccessful.

INTRODUCTION

Demodicosis in sheep caused by the mite *Demodex ovis* Raillet (1895) was first recorded as far back as 1842 when Simon (cited by Nemeséri & Széky[1]) found the mites in the meibomian gland of the upper eyelid. Ten years later Oschatz confirmed this observation and the occurrence of this mite in sheep has been studied by various workers subsequently[3,4,5]. Nemeséri and Széky[1] have reviewed the literature and quote the following reported distribution: Amiel in 1929 and Aynaud in 1931 stated that demodicosis in sheep is of frequent occurrence in certain areas in France, Algeria, Morocco and occurs in Spain, Turkey, Argentinia and India. Outbreaks have been recorded in England by Hirst in 1919 and by Brownlee in 1935, in Australia by Carter in 1942, in Germany by Klein in 1922 and by Opperman in 1950, in the U.S.A. by Baker and Nutting in 1950 and in the Soviet Union by Klinskyi in 1957.

According to Nemeséri & Széky[1], demodicosis in sheep may be regarded as a mild infestation comparable to that in man where there is little tendency towards the formation of nodules, the condition usually assuming the squamous form characterised by little or no pruritis and only a very mild degree of keratinization. According to Hirst[2], Klein described a serious form of the disease in two large flocks in Germany. In one flock of six hundred sheep nearly every animal was affected, the fleeces being torn or hanging loose in places. In advanced cases the animals were in moderate physical condition but the wool had disappeared from the flanks, back, neck and sides of the body and the skin became as hard as a board, thickened, and strongly wrinkled in the region of the neck. In badly affected cases pruritis was intense and *Demodex* mites were present in large numbers. Hirst states that the symptoms described are suggestive of a mixed infection with possibly *Sarcoptes* or *Psoroptes*.

That demodicosis has as yet not been recorded from South Africa is possibly due to the fact that the infestation in sheep is of a mild nature and may have escaped detection.

The object of the present paper is to place on record the occurrence of an infestation with *Demodex ovis* in a single Merino sheep in a flock of several hundred in the Standerton district of the Transvaal.

MATERIAL AND METHOD

During the course of a routine inspection of sheep for the presence of Infectious Itch (*Psorergates ovis* infection) skin scrapings were made from a number of sheep which showed a greater or lesser degree of disturbance of the fleece.

One sheep, which showed a few locks of wool partially detached from the fleece, was subjected to close scrutiny after an unsuccessful attempt to demonstrate the presence of *Psorergates* mites. Skin scrapings were made after the wool had been closely clipped over several areas of roughly two square inches situated on the sides of the body, 1-2 cc of liquid paraffin having been rubbed into the skin prior to scraping. Upon examination under a steroscopic dissection microscope, numerous rather slender demodectic mites in all stages of development were observed (Plate 1).

On opening the fleece, the skin over the back and sides of the body was diffusely reddened with a purplish hue. No thickening of the skin could be detected on palpation. Over a few isolated areas of about one inch in diameter the normal growth of wool had been affected, resulting in more or less complete breaks in the continuity of the fibres; these had become detached from the fleece, resulting in the protruding locks previously mentioned. Wool growth had continued, how-

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ever, on these areas but the newly grown wool, although normal in appearance, was considerably shorter than the remainder of the fleece.

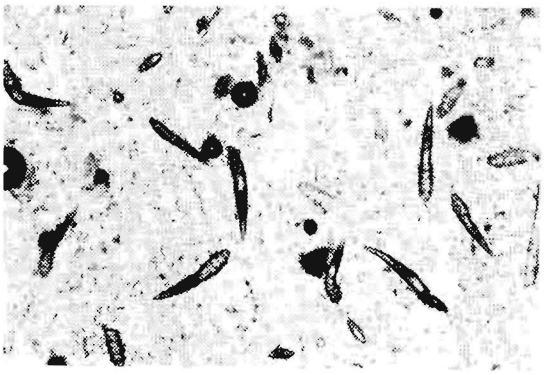

Plate 1 — Skin scraping with demodectic mites in various stages of development.

To ascertain the degree of infestation and involvement of dermal tissue, a skin biopsy was taken from the thoracic region for sectioning and microscopic examination.

Hair follicles showed the presence of numerous demodectic mites which were also present in limited numbers in the sebaceous glands. A moderate degree of round cell infiltration was present together with accumulations of erythrocytes which accounted for the diffuse erythema of the skin. In spite of the large number of demodectic mites, skin reaction was present to a moderate degree only.

Transmission was attempted in the following ways:

1. The infested sheep was shorn on one side only and the shorn side was placed into contact with the side of a freshly shorn uninfested sheep, the two sheep being held in this position in a narrow cage overnight to ensure as close contact as possible and to simulate conditions on farms where, after shearing, sheep are forced into close contact with each other by being herded into a small holding kraal, frequently for several hours. It has been assumed that under such conditions the infestation spreads from sheep to sheep.

2. Scrapings taken from two areas on the infested sheep were transferred to the skin of an uninfested sheep, the liquid scrapings being rubbed into the skin by means of the fingers. Liquid paraffin was used as a vehicle in the one case and water in the other.

These attempts at transmission were continued over a period of three months but proved entirely unsuccessful.

Conclusions

From the foregoing account it may be concluded that, under normal conditions demodectic mange in sheep may be regarded as a diffuse infestation of a mild nature with only a moderate degree of skin reaction. Infectivity appears to be of a low grade as only a single case was detected in a flock of several hundred after examination of some ten sheep which showed disturbance of the fleece. Furthermore, attempts at transmitting the infestation by close contact after shearing and mechanically transferring large numbers of mites proved unsuccessful.

ACKNOWLEDGEMENTS

We would like to express our appreciation to Dr. R. Tustin of the Dept. of Pathology who undertook the sectioning and examintaion of the skin biopsy specimens; the Chief, Onderstepoort Institute for Veterinary Research for permission to publish in this journal.

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DESTINATION OF SALMONELLA CONTAMINATED FOODS AND FEEDS

Report of the Round Table Conference, organized by the World Association of Veterinary Food-Hygienists.

May 3, 4 and 5, 1967

BILTHOVEN, The Netherlands

Importance of the Salmonella problem on a world-wide scale, with particular reference to the destination of materials found contaminated.

1. *EPIDEMIOLOGY*

Human Salmonellosis

The morbidity of salmonellosis is estimated, depending on the degree of perfection of the reporting system, at 10 - 1000 times the figures occurring in the official epidemiological reports. Surveillance and reporting are indeed extremely poor in some areas of the world: unless human salmonellosis is made a mandatory notifiable disease, no accurate assessment of the morbidity is possible.

In all countries the predominant serotype in human morbidity is *S. typhi murium.* The frequency of other serotypes, such as *S. panama, S. heidelberg, S. enteritidis* depends upon the type of food and feed used in a certain area and may vary widely: some of these serotypes reach epidemic proportions and may remain at high levels. *S. paratyphi B* and *S. typhi* are decreasing everywhere.

Severe cases of salmonellosis are observed primarily in the age-groups less than 1 year and over 60 years. The mortality is also highest in those groups.

Red meat, poultry, eggs and their products play the most important role as causes of salmonellosis. The particular type of food that is the most important vehicle, depends on the food preparation habits in a given region. The mere occurrence of salmonellae in a particular food will, therefore, not always be reflected in the epidemiology of human salmonellosis.

Contaminated water may be a source of salmonellae, particularly when potable water is not used exclusively in food processing plants, for the cooling of canned foods, and in animal husbandry.

Although direct transmission of salmonellosis in nurseries and hospitals has been described, it is not an important mode of spread of salmonellae in the general population.

The salmonella carrier rate in the human population in developed areas is seldom higher than 0.3%; these human excretors are potential sources of food contamination. Systematic bacteriological examination of stools is generally considered useful only for the follow-up of known carriers of *S. typhi* and *S. paratyphi B,* and for new employees; the detection of carriers of other salmonella serotypes is burdensome and ineffective under practical conditions.

Salmonella in animals

Clinical salmonellosis occurs not infrequently in all kinds of animals slaughtered for food, particularly in young creatures. However, this may be relatively unimportant in human salmonellosis, because clinically sick animals will be eliminated by Veterinary Meat Inspection. But meat from sick animals given to other animals may be an indirect cause of human salmonellosis.

The incidence of salmonella carrier animals varies greatly with the mode in which the animals are raised and held, but generally it is alarming. Slaughter of such clinically healthy animals which excrete salmonellae in their faeces is a serious problem, in all areas of the world, because such animals cannot be detected by the usual meat inspection techniques.

It has been demonstrated almost everywhere that feeds play an important role in the spread of salmonellae in farm animals. Bacteriological studies on bone-, meat- and blood-meal, meals of vegetable origin, such as soya-scrap, cotton-seed and sunflower-seed, and fish-meal have revealed that these substances are the main sources of salmonellae in mixed feeds. By-products of food processing plants, such as poultry and meat offal and of certain dairy plants, which are returned to the farms have repeatedly led to

animal salmonellosis when the material has not been decontaminated properly before leaving the factory.

On farms where salmonella carriers are present or clinical salmonellosis occurs, the environment may influence the spread of salmonellae over long periods of time. In addition, new contamination of the herds and flocks may occur through wild birds, flies, rodents or other animals, water, man, and air-borne particles. Environmental sanitation is therefore of utmost importance; however, complete control of environmental contamination on farms has been proved to be extremely difficult to attain.

Slaughtering plays a major role in the spread of salmonellae. Imperfect transportation, the holding of large numbers of animals in pens and modern large-scale slaughter processes, where great numbers of animals pass the line continually for many hours, leads to cross-contamination with salmonellae, even when stringent measures of sanitation are taken. Continuous surveillance and improvement of the bacteriological condition of the killing-floor, the dehairing machines used for pig carcasses, the removal of feather and chilling processes in poultry slaughter, the equipment and of the hands of the staff handling meat, has resulted in the reduction, but not in the prevention of cross-contamination. Further work in this area should be encouraged. However, it is unlikely that full control of cross-contamination can be achieved unless considerable advances are made in the reduction of the number of salmonella excretors amongst animals offered for slaughter.

2. *ECONOMIC SIGNIFICANCE OF SALMONELLOSIS*

Direct economic losses due to human salmonellosis may be estimated at approximately $50 (U.S.A.) per patient. In addition, there are considerable overhead expenses involved in the maintenance of Public Health Services at city, country and national level.

Except in a few countries, importation requirements with regard to salmonellae for foods and feeds have not yet incurred much economic loss to the importing trade. However, it is likely that more stringent inspection measures will be established, whereupon financial complications may be expected, both with regard to the costs of laboratory investigations and the rejection of contaminated material.

Contrary to the situation at importation level, significant financial losses have already occurred due to repeated rejection of consignments destined for exportation. Where it seems impossible to remedy the situation, loss of markets is inevitable.

3. *PREVENTION*

Because of the multiplicity of hosts, total eradication of human salmonellosis is impossible at present. Hence, the primary objective in the control of human salmonellosis is to continue efforts to produce salmonella-free foods of animal origin. This requires, primarily, the raising of salmonella-free herds and flocks. There are indications that salmonella-free animals may be obtained by a combination of decontamination of feeds and control of the environment at the farm. Until the attainment of virtually salmonella-free animals is possible, a high standard of sanitation should be maintained on rearing and production farms, during transport, in holding pens, during slaughter, in meat processing plants, butcher shops, super-markets, restaurants and homes.

Furthermore, attempts to reduce salmonellosis morbidity without paying proper attention to the bacteriological quality of imported foods is a fruitless procedure.

In all salmonella control programmes, the standardization of methodology is important both with regard to determining the incidence of contamination and also for evaluating physical and chemical measures of decontamination.

While no guarantee exists that most foods in the trade are salmonella-free, education of the producers, the trade and the consumers with regard to hygienic handling, cold-storage, adequate heating of potentially contaminated products prior to consumption, and the hazards of cross-contamination from raw to cooked products via surfaces, equipment and hands is essential.

4. *LEGISLATION*

Effective bacteriological control exists for very few imported food products. The situation is far worse for imported feed components.

Also, legislation with regard to exportation of foods, other than the national legislation, exists only in a few countries.

5. *POLICY WITH REGARD TO ACCEPTANCE OF CONSIGNMENTS OF FOOD AND FEED*

The distribution of salmonellae is heterogeneous in most foods and feeds. The examination of food and feed samples that have been taken without attention to their adequacy in representing the consignments from which they are derived, may therefore, be not only wasted effort, but also dangerous in conferring an unjustified feeling of security to the trade.

Because of difficulties of sampling and analysis, a negative result for salmonellae is not sufficient guarantee of the absence of the organism. For this reason, importing nations should consider either a compulsory decontaminating process known to be effective, or a test for microorganisms known to indicate insanitary practice.

Standardization of sampling procedures and of bacteriological methods of examination is a prerequisite for comparison and cooperation between countries. When buyer and seller do not use the same methods, serious difficulties have occurred and are to be anticipated again.

At present enormous amounts of salmonella-contaminated foods and feeds are traded around the world and no immediate reduction in the contamination rate is to be expected. Hence, the introduction of a system of acceptance based on increasingly stringent criteria for rejection should be seriously considered as a first step in attempts to control the situation. The priority to be given to the various commodities and the limits of their acceptability will depend on epidemiological evidence.

6. *DESTINATION OF REJECTED CONSIGNMENTS*

Modes of decontamination

Since salmonellae are easily eliminated by heat, the destruction of salmonella-contaminated goods will seldom be required. Denaturation is practiced in some countries, but it is not to be recommended, since it will not effectively break the cycle of salmonella contamination.

Decontamination by heat is, and will be for the immediate future, the most efficient mode of treating substandard consignments. The heat treatment of moist materials is always effective when carried out for about ten minutes at temperatures of about 70°C. Decontamination of dry goods requires either much higher temperatures, which may lead to losses of nutritive value, or pre-moistening of the material to a water activity of at least 0.75, in which case they can be treated as moist foods and there is no loss of nutritive value.

Irradiation of foods and feeds with gamma-rays for salmonella decontamination has not yet been applied commercially. According to the results of experiments this procedure is most promising and could be important in the future. For the decontamination of feed ingredients and of mixed feeds, irradiation is one of the few possible methods of treatment, the production of pellets an attractive alternative. For frozen meats and poultry irradiation is at present the only process that secures reliable decontamination without changing the character or the wholesomeness of the food.

Chemical methods for decontamination, such as fumigation with ethylene oxyde or beta-propiolactone, and the addition of certain acids with bactericidal properties, have occasionally been applied experimentally. It seems that some of these processes give rise to toxicological or technological difficulties.

Checks of efficiency after decontamination

The occurrence of salmonellae in contaminated foods and feeds is sporadic and heterogeneous. The mode of distribution of these bacteria in decontaminated products is, obviously, much more so, which makes their detection virtually impossible. Instead quantitative tests for low numbers of surviving *Enterobacteriaceae,* or other appropriate indicator organisms, should be applied.

RECOMMENDATIONS

1. Salmonellosis is a serious public health problem on a world-wide scale, affecting millions of persons. It also creates important economic problems.
2. National Salmonella Surveillance should be inaugurated as soon as possible in countries where it does not presently exist. The programme should be a joint effort of public health and animal health services, and coordinated with the W.H.O. International Salmonella Surveillance.
3. An internationally acceptable system of sampling and laboratory methodology for the isolation of salmonellae, suitable for all pertinent materials including foods and feeds must be developed and tested collaboratively. Formal training for microbiologists in these procedures should be provided.

4. A primary responsibility of the food and feed industries and of the relevant government agencies is to eliminate salmonellae from food and feed products generally known or suspected to be implicated in human and animal disease outbreaks.
5. Reduction in the incidence of salmonellae requires better animal practices of which essential elements are improved animal husbandry, avoidance of overstocking and movement of very young stock, and the control of the bacteriological condition of mixed feeds. In connection with these measures the control of salmonella infections in flocks and herds should be based on sound epidemiological investigations. In some countries success has been achieved with the use of salmonella controlled feed, and preventive veterinary medical practices.
6. Animals in transport and in lairages should not be overcrowded, and should not be held for long periods. Lairages should be sanitary. To reduce contamination of meat products with salmonellae the following points are important in abattoirs and meat processing establishments: Separation of "clean and unclean" departments; the maintenance of a high standard of cleanliness throughout the abattoir; proper lay-out of the killing and processing line; hygienic handling and processing of meat; and the provision of hygienic facilities for plant personnel.
7. Public health education in meat and food hygiene should start in primary schools and continue in technical colleges. The aim should be to educate all food handlers and processors, who ought to be aware of methods for the prevention of salmonellosis. Consumers should be informed that foods of animal origin must be stored at temperatures that will prevent the growth of salmonellae before and after preparation and that raw products must be heated sufficiently to kill these organisms.
8. Selection of methods for the examination of food and feed samples should be based on comparative tests carried out internationally. If possible, sampling should be based upon statistical principles which will permit the expression of calculated limits of acceptance. Increasingly stringent criteria for rejection should be instituted. The limits of acceptance should be considered in relation to the following factors:
   a. The importance of a particular product, considering the nutritional or economic priorities within the country concerned.
   b. The national salmonella situation; for example, where the incidence of salmonellosis is low, limits of acceptability should be strict.
   c. Specific epidemiological evidence that a particular product is especially dangerous.
   d. Processed manufactured products which may be subject to recontamination should be more rigidly controlled than raw products.
9. Imports of essential food and feed products, when found to be contaminated with salmonellae should be regularly examined until a decision is reached, whether to:
   a. discontinue importation from the country which does not show the necessary improvement in hygienic standards;
   b. institute some obligatory form of treatment such as heating, pelleting, solvent extraction or, when permitted, irradiation to prevent loss of food or feedstuffs and to facilitate distribution.
10. Certificates which guarantee that foods and feeds are free from salmonellae are misleading, because in the present stage of the art of production and processing of certain foods and feeds, it is impossible to guarantee the absence of these organisms. Also, importing countries should not exert pressure on the authorities in exporting countries to issue such certificates. If, nevertheless, certificates relating to salmonellae are used, they should (i) describe the metohds of processing, if any, that have been used to destroy these organisms; (ii) give details of the techniques used for sampling and examination; and (iii) the results obtained.
11. The effect of severe trade restrictions on the need for protein foods and on the food industry should be seriously considered. The world need for food increases annually with the expanding human population hence it is necessary to salvage food.
12. In view of the hazard of salmonellosis to patients and hospital populations particular attention should be paid to proper procurement of safe foods and the highest standard of sanitation in the preparation and service of meals in hospitals. The same precautions should be taken in the preparation and service of food in nurseries, kindergartens and homes for the aged.
13. The Salmonella Committee of the W.A.V.F.H. recommends to WHO/FAO that an expert advisory panel on salmonellosis be established to study and advise on this world problem.

ANTHELMINTIC AND TOXICITY STUDIES WITH TETRAMISOLE. II TOXICITY STUDIES IN SHEEP AND GOATS.

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SUMMARY

Acute toxicity studies have shown that tetramisole hydrochloride is entirely safe for sheep and Angora goats at dosage levels of up to 60 mg/kg. Occasional deaths occurred in Angora goats at 75 mg/kg (5 times the therapeutic dose) and in sheep at 90 mg/kg (6 times the therapeutic dose). Animals with pre-existing liver damage appear more susceptible and occasional deaths in sheep were observed at 75 mg/kg.

Pregnancy, dipping in an organic phosphate, or journeys by rail or road transport did not increase susceptibility to any toxic effects.

Repeated dosing at twice the recommended dose, every 2 weeks for periods up to 7 months, did not enhance toxicity or give rise to weight loss, infertility or abortions amongst Merino sheep or Angora goats.

A total of 48,739 sheep and goats, on 59 farms throughout the Republic of South Africa and South West Africa, under varying conditions of management, were dosed at double the recommended use rate. A total of 32 (0.66 per cent) sheep died within 3 days after dosing, and 44 showed specific transient side-effects. Of these deaths, only a small proportion could be ascribed to the direct effects of tetramisole toxicity. There were no deaths amongst 5,588 Karakul sheep and 2,576 Angora goats dosed.

INTRODUCTION

The development of new anthelmintic compounds requires not only an appraisal of efficacy against a wide range of mature and immature worm species but also an evaluation of their safety under a variety of conditions most likely to be met in the field. The study reported here was undertaken to determine the safety under practical farming conditions of the anthelmintic tetramisole hydrochloride (Ripercol**), in sheep and goats prior to its commercial release in the Republic of South Africa and South West Africa.

Acute and chronic toxicity studies may be conducted under controlled conditions in the laboratory whereas field toxicity studies involve the administration of the product to large numbers of different species and breeds in many different geographical regions under varying climatic, management and nutritional conditions.

Walley[1] in Great Britain and Forsythe[2] in Australia have reported on similar toxicity studies with tetramisole conducted in those countries.

A. ACUTE TOXICITY STUDIES

MATERIALS AND METHODS

Tetramisole: Aqueous solutions of tetramisole hydrochloride were prepared as 1, 3 or 6 per cent w/v concentrations depending on the volume to be dosed. All dosage rates were based on the hydrochloride salt and individual doses were calculated from the actual liveweight of the animals at the time of dosing, irrespective of wool or hair growth, rumen fill etc.

Mode of Administration: Tetramisole solution was administered into the back of the mouth with a 'Phillips' 10 cc, automatic or 'Neelino' 30 cc. dosing gun with the exception of three animals where the dose was injected directly into the dorsal ruminal sac with a hypodermic needle.

Animals: A total of 280 animals consisting of Merino and German Merino ewes, Dorper and Blackhead Persian cross lambs and purebred Angora kids and ewes were used in these studies which extended over a period of approximately 3 months. Many of these animals were used in more than one trial during this period.

Feed and Management: The sheep and goats were varyingly fed teff hay, lucerne hay or very poor quality veld hay with and without supplementary cubed feed. Some groups were maintained on concrete during the entire period, others allowed to graze on average poorly fertilized Kikuyu pastures during the day. For security reasons, all animals were housed at night.

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**Registered trademark of Janssen Pharmaceutica, division of Ethnor (Pty.) Ltd., South Africa.

Records and Observations: All animals were kept under continuous observation for 3 to 4 hours following dosing and at regular intervals thereafter. Detailed records were maintained of symptoms of toxicity and general behaviour following dosing. All animals that died were subjected to post-mortem examination.

RESULTS

A summary of the data on sheep (excluding *Fasciola gigantica* infested ewes) dosed with tetramisole at various dosage rates and the results of such dosing is presented in Table 1.

fested with 100 *Fasciola gigantica* metacercariae. Two of these ewes died as a result of acute fasciolasis prior to dosing with tetramisole and in common with those that died following dosing, showed acute liver degeneration with haemorrhage caused by large numbers (80 - 100 in most cases) of flukes.

The fully lethal dose for sheep is probably in the region of 100 mg/kg bodyweight. A dose of 90 mg/kg (six times the therapeutic dose) resulted in the death of 3 out of 11 animals dosed, and 2 out of 5 in the case of sheep with severely damaged livers.

TABLE 1.—ACUTE TOXICITY STUDIES.

Summary of the results on the total number of sheep dosed (excluding *Fasciola gigantica* infested ewes) at varying rates with tetramisole hydrochloride.

Dose mg/kg bodyweight	Number Dosed			Mortality			Symptoms Only		
	Ewes	Lambs	Total	Ewes	Lambs	Total	Ewes	Lambs	Total
105 (7xTD–)	0	1	1	0	1	1 (100%)	0		0
90 (6xTD)	3	8	11	1	2	3 (27%)	2	6	8 (73%)
75 (5xTD)	24	33	57	0	0	0	0	7	7 (1.2%)
60 (4xTD)	27	5	32	0	0	0	2	2	4 (12.5%)
45 (3xTD)	0	138	138	0	0	0	0	1	1 (0.72%)
30 (2xTD)	153	1	154	0	0	0	0	0	0

–TD = Therapeutic dose (15 mg/kg bodyweight).

Table 2 summarizes the results obtained in an experimental flock of adult German Merino ewes each of which had previously been artificially in-

TABLE 2.—ACUTE TOXICITY STUDIES

Results of dosing a flock of *Fasciola gigantica* infested ewes at various rates with tetramisole hydrochloride.

Dose mg/kg *bodyweight*	Number *Dosed*	Mortality *(No.)*	Symptoms *(No.)*
90	5	2	2
75	3	1	0
60	16	0	1
30	20*	0	0

*Dosed twice (30 mg/kg) at 14 days interval.

A few individual sheep exhibited marked toxic symptoms at a dose of 75 mg/kg but no mortality occurred except in one sheep where prior liver damage was present.

After sub-lethal dosing, transient side-effects were seen in the majority of animals dosed at 90 mg/kg and in varying number at lower dosage rate. Symptoms generally commenced within 5 - 10 minutes after dosing and consisted of head-shaking, mouth chewing movements, salivation, intermittent muscular tremors, ataxia, hyperpnoea, nervous irritability and frequent urination and defaecation. Occasional animals showed crouching and short jumping movements. Depending on the initial degree of severity these effects gradually

diminished over a period of from 1 to 6 hours, after which no further symptoms were noted.

Following a single lethal dose accentuated symptoms were seen followed by chest recumbency and respiratory failure which preceded cardiac arrest. Death occurred within ½ - 2 hours after drenching.

Autopsy invariably failed to show any specific lesions which could be constantly attributed to the effects of the drug. Lesions associated with unrelated concommitant conditions were noted in a few cases, e.g. fasciolasis, corynebacterium infection, etc.

Effect on Pregnant Ewes: Many of the adult ewes dosed were 3½ to 4 months pregnant. No enhanced effects, abortions or still-births were observed amongst these animals.

Simultaneous dosing with a cesticide: A group of 60 cross-bred Merino lambs averaging 30 - 35 lb bodyweight were dosed with tetramisole at a dose rate of 45 mg/kg immediately followed by a dose of niclosamide at approximately 100 mg/kg. No symptoms of toxicity or increased peristaltic action were noted.

Simultaneous Dipping in Organo-Phosphate Compound: A group of 50 pregnant Merino ewes were dipped by individual immersion in a drum containing 0.03% diazanon, the volume being regularly replenished with 0.05% emulsion. Each animal was then immediately dosed with tetramisole at the rate of 30 mg/kg bodyweight.

No symptoms of toxicity were observed and there were no abortions or still-births.

Intra-Rumenal Administration: Three mature ewes to which tetramisole (3% solution) was administered at the rate of 60, 75 and 90 mg/kg respectively, by direct intra-rumenal injection, showed no symptoms of toxicity.

Effects of Transportation Stress: A flock of 50 Merino ewes in poor condition purchased in the Bethlehem district, was transported by road to the research station in Kempton Park (approximately 200 miles). Immediately on arrival they were weighed and dosed with tetramisole at the rate of 30 mg/kg bodyweight. No symptoms of toxicity were observed.

A flock of 20 pre-bred Angora ewes, mostly pregnant, was purchased in the Eastern Cape Province and transported by rail to the research station (a 4½ day journey). Immediately on arrival they were weighed and dosed with tetramisole at the rate of 30 mg/kg bodyweight. There were no symptoms of toxicity. Six days later the same flock was dosed at the rate of 75 mg/kg. Ten animals showed marked nervous symptoms and two of these died within 30 minutes. The remainder recovered within a period of 6 - 8 hours. However, three days later one goat developed inappetence and rumenal atony, became semi-comatose and died within 12 hours. Post mortem lesions were obscured by advanced decomposition.

A group of ten 9-month old Angora kids, after a 4-day train journey from the Cape Province, was dosed with tetramisole at the rate of 30 mg/kg immediately following arrival. No side-effects were noted and the kids were dosed again one week later at the rate of 75 mg/kg. No severe symptoms were noted and no losses occurred.

The data on acute toxicity studies in Angora goats is summarized in Table 3.

TABLE 3.—ACUTE TOXICITY STUDIES

Summary of the results on the Angora goats dosed at varying levels with tetramisole hydrochloride.

Dose mg/kg bodyweight	Number Dosed			Mortality			Symptoms only		
	Ewes	Kids	Total	Ewes	Kids	Total	Ewes	Kids	Total
75 (5xTD–)	20	10	30	2	0	2	8	0	8
30 (2xTD)	20	10	30	0	0	0	0	0	0

–TD = Therapeutic dose (15 mg/kg).

B. CHRONIC TOXICITY STUDIES

Trial 1:

MATERIALS AND METHODS

A flock of 21 mature German Merino ewes which had previously been artificially infested with *F. gigantica* metacercariae was divided randomly into two groups. One group consisting of 10 ewes was dosed with tetramisole (6% solution) at the rate of 30 mg/kg every 14 days for approximately 7 months, a total of 15 doses being administered during this period. The remaining 11 ewes were not treated. Both groups were housed in concrete floored pens.

A German Merino ram, weighing approximately 190 lb. was introduced into the experimental flock at the start of the trial and dosed with tetramisole every two weeks, also at the rate of 30 mg/kg bodyweight. During this period of the trial this ram was also used to serve another flock of 23 (untreated) ewes.

The trial was terminated 31 days after the last lamb had been born. Initial and terminal bodyweights were recorded and the animals closely observed for clinical symptoms following each administration of tetramisole.

RESULTS

(a) *Mortality*: During the period of the trial, two ewes in the treated group died, one as a result of acute fasciolasis, the other following dystocia. In both cases the interval between tetramisole administration and death was at least seven days. No symptoms of toxicity were seen in any of the sheep following dosing. No mortality occurred amongst the control group.

(b) *Lambing percentage*: Although the lambing percentage was low in both experimental groups, 60% in treated ewes and 45% in controls, the same ram was also used to cover a separate flock of 23 ewes from which 21 lambs were born. There was therefore no detectable effect on fertility in either the treated ewes or the ram. Lambs were not weighed at birth but appeared comparable in size and subsequent growth rate to lambs from untreated ewes.

(c) *Bodyweights*: Over the seven month test period, the treated group lost an average of 19.5 lb bodyweight per sheep against an average loss of 14 lb in the controls. Due to wide variations between individual sheep in both groups, the difference in average weights is not significant. In both cases the loss is attributed to the fact that the lambs had not yet been weaned by the time the trial was terminated.

Trial 2:

MATERIALS AND METHODS

A flock of 50 very old Merino ewes with an initial average bodyweight of 85 lb was randomly divided into two equal groups and housed in covered pens with concrete floors. One group was dosed every second week with a 6% solution of tetramisole at the rate of 30 mg/kg for a total period of 7 months, while the remainder were untreated. Initial and terminal bodyweights were recorded and visual observations made for clinical signs of toxicity.

RESULTS

Two sheep in the untreated group died during the course of the trial but no mortality occurred amongst the group receiving tetramisole, neither were symptoms of toxicity observed at any time. No significant change in mean liveweight occurred in either group.

Trial 3:

MATERIALS AND METHODS

A small flock of Angora goats consisting of 13 ewes and 8 kids was used to study the effects of repeated dosing with tetramisole at fortnightly intervals over a period of 6 months. Seven ewes and 4 kids were dosed at the rate of 30 mg/kg and 45 mg/kg bodyweight respectively, the remainder of the flock remained untreated. Five ewes in the treated group were pregnant at the commencement of the trial.

Comparative observations were made with regard to liveweight gains and clinical symptoms.

RESULTS

Amongst the ewes there was an average net weight gain of 4.5 lb in the treated, versus 6.6 lb in the control group over the total period, while the kids gained 17.6 lb and 15.4 lb respectively. No mortality occurred in any of the groups but occasional symptoms consisting of head shaking, sneezing and slight salivation were noted amongst the kids immediately following dosing. There were no abortions amongst pregnant ewes.

C. FIELD TOXICITY STUDIES

MATERIALS AND METHODS

Tetramisole Solution: Solutions were prepared by mixing 268 grams of pure tetramisole hydrochloride in 1 gallon of water (6 per cent) or 134 grams per gallon (3 per cent). The stronger solution is more convenient for use in automatic dosing guns and was used in the majority of trials. Solutions were prepared from municipal, borehole or dam water supplies as available in the local area.

Method of Administration: In all cases the dose was administered into the back of the mouth using a 'Phillips' 10 or 15 cc automatic dosing gun. These guns were tested in our laboratory and found to be extremely accurate. No mouth gags were used.

Dose rates: Since it was impractical under field conditions to dose each sheep on the basis of exact bodyweight. 15 - 20 of the larger and smaller animals within a given age group were indi-

vidually weighed on a portable scale to establish the weight range and the dose volume to be used. It will be seen from Table 4 that a proportion of animals within a group might well receive almost three times the normal recommended dose of 15 mg/kg.

TABLE 4.—FIELD TOXICITY STUDIES.
Field toxicity test doses of tetramisole hydrochloriede for different weight groups of sheep and goats.

	Field Toxicity Test Dose.	
Liveweight (lb)	Volume (6% solution)	Range (mg/kg)
Under 30	5 cc	22.7 — (±) 40
30 —50	10 cc	26.4 — 44.1
51 —75	15 cc	26.4 — 38.8
Over 75	20 cc	34.8 — <17.6

Records and Observations: Immediately following dosing, the flock was observed for at least one hour and retained in the kraal or nearby camp for 4 - 6 hours. Any animals showing marked side-effects were generally kept aside for a period. Approximately 3 - 5 days following dosing, it was ascertained whether subsequent losses or abnormal behaviour had been observed in the flock.
Sheep and Goats Dosed: Trials were conducted with a variety of animals consisting of Merino, Karakul, Blackhead Persian, German Merino, Dorper, Damara and cross-bred sheep, Angora and Boer goats. Mature, young, emaciated, fat, pregnant and recently lambed animals were dosed. Farms were also selected where seneciosis, lupinosis and geeldikkop were known to occur. Many sheep and goats were carrying up to 10 months wool or hair, others had been recently shorn. Some flocks had been deprived of food and water for up to 48 hours in unshaded pens prior to dosing, and others had been trekked up to 10 miles. Climatic conditions varied from extreme drought-stricken inland areas to high-rainfall coastal areas with lush winter pastures.

RESULTS

A summary of the results showing the number and location of farms and the type and numbers of animals dosed, is given in Table 5. When these results are analysed on the basis of weight range (Table 6), it will be noted that almost half of the total number of animals dosed were between 50 - 75 lb bodyweight and that this group also suffered the highest percentage mortality.
Symptoms of Intoxication: In general, the side-effects seen in 0.09% of animals following tetramisole administration were milder and of shorter duration than those seen during acute toxicity studies on our experimental unit. In addition, a number of animals were seen to shake their heads, and goats in particular were inclined to sneeze for approximately 3 - 5 minutes following dosing.
Post Mortem Examinations: Due to long distances between farms it was only possible for post mortem examinations to be undertaken by veterinarians on 14 of the 32 sheep which died during the field toxicity studies. In 3 of the sheep dying on one farm, post mortem lesions differed from those seen during acute toxicity studies in that marked sub-epicardial haemorrhages and intense inflammation of a portion of the small intestine were noted in all 3 animals. Two of 3 animals which died on another farm were reported to have an acute liver degeneration with marked sub-capsular haemorrhages. The sheep were in very poor condition.

Two sheep which died on another property in the same area (Karroo) were found to be suffering from extensive corynebacteriasis involving the lungs, liver and associated lymph nodes. Post mortem examination of seven sheep that died within three days following dosing on a property in the Orange Free State showed a marked accumulation of fluid in the chest and abdominal cavities. The livers were abnormally hard with histological evidence of interlobular cirrhosis. A differential diagnosis of chronic seneciosis could not be ruled out as this plant was known to be present on the farm.

Thirteen animals on one farm died 24 to 36 hours following dosing but were not autopsied. The owner reported seeing a frothy discharge from the mouth and nostrils.

DISCUSSION

The acute toxicity studies were not designed to determine the exact LD for tetramisole in normal sheep or lambs. From the data obtained, however this figure probably lies somewhere between 90 - 100 mg/kg. Limited laboratory studies indicated that sheep suffering from an acute liver derangement may be more susceptible to the effects of tetramisole and this finding was supported by field toxicity studies.

Superimposed stress conditions such as long journeys by rail, or on the hoof, simultaneous dipping in an organo-phosphate or dosing with niclosamide, starvation or water deprivation did not appear to predispose sheep or goats to toxicity. Frequent dosing, every 3 - 4 weeks over a long period may conceivably be practised in some areas where intensively grazed ley pastures are utilized. No toxic or cumulative side-effects such as loss of weight, abortion or lowered fertility were noted in sheep or goats subjected to fort-

TABLE 5.—SUMMARY OF THE RESULTS OF DOSING SHEEP AND GOATS AT TWICE THE RECOMMENDED USE DOSE OF TETRAMISOLE HYDROCHLORIDE IN VARIOUS AREAS OF SOUTH AFRICA AND SOUTH WEST AFRICA.

Area	Number of farms	Merinos	Karakuls	Black head Persians	Cross-breds and Dorpers	Angora goats	Boer goats	Suffolk and Crosses	Sub-Totals	Mortality	Per cent
Eastern Cape Province and Border.....	17	8,652	—	—	35	2,563	178	—	11,428	17	0.148
Western Cape Province.....	6	4,444	—	—	744	—	—	—	5,188	0	0
Karroo.....	11	6,257	—	—	512	—	16	—	6,785	5	0.074
Orange Free State	5	5,104	—	—	—	—	—	—	5,104	7	0.137
Transvaal,.....	6	6,766	—	—	—	—	—	—	6,766	1	0.015
Natal and East Griqualand....	7	6,373	—	—	—	—	—	371	6,744	2	0.029
South West Africa	7	36	5,588	1,001	—	13	86	—	6,724	0	0
SUB-TOTAL....	59	37,632	5,588	1,001	1,291	2,576	280	371	48,739	32	0.066

TABLE 6.—ANALYSIS OF ANIMALS SHOWING SYMPTOMS OR DYING FOLLOWING ADMINISTRATION OF TETRAMISOLE HYDROCHLORIDE AT TWICE THE RECOMMENDED USE DOSE IN RELATION TO BODYWEIGHT.

Weight Range (lb)	Dose (cc)	Total Animals Dosed	No. showing symptoms	Mortality	
				Total	Per cent
Under 30.....	5 cc (6%) or 10 cc (3%)	3,786	3	0	0
30—50.....	10 cc (6%) or 20 cc (3%)	7,820	10	4	0.05
50—75.....	15 cc (6%) or 30 cc (3%)	23,303	19	21	0.09
Over 75.....	20 cc (6%) or 40 cc (3%)	13,830	12	7	0.05
TOTAL.....		48,739	44	32	0.066

nightly dosing at double the recommended level of tetramisole hydrochloride.

The possibility of sheep receiving a double dose is quite likely under farm conditions and particularly where old-fashioned inaccurate drenching equipment is used it is possible that a small number (1 - 2%) would show severe toxic symptoms. From our experience occasional deaths are likely to occur where such sheep are predisposed to toxic effects by pre-existing liver damage or other debilitating conditions. The occasional lip licking, head shaking or sneezing seen immediately following dosing did not cause concern from the owner's point of view and were not recorded as side effects. In some flocks a few animals with muscular tremors, ataxia or excitement caused concern initially but these appeared to recover fully within 3 - 4 hours.

The ease of drenching with a product that is completely soluble together with the small dose required makes it ideal for use in automatic dosing guns. Provided the solution is kept free of dirt, no problem is experienced with sticky valves or faulty dose delivery. Flock owners were particularly impressed with this aspect since two operators were able to dose up to 1,200 sheep an hour with a minimum of additional helpers or manhandling.

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PATTERN OF THE OESTROUS CYCLE OF MARES

I. The breeding season.

C. H. VAN NIEKERK*.

SUMMARY

The breeding cycles of 188 mares of three different breeds are summarized.

Although mares were found to be polyoestrous with a tendency to become monoestrous the optimum breeding season for more than 90% of the mares under observation was found to be in summer (November to February).

Ninety-six per cent of oestrous periods occurring during summer ended in ovulation. Only 19% of mares in spring oestrus ovulated. Although the percentage conception rate of served mares remained fairly constant throughout the year, the percentage of fertile services was much higher during summer (52%) than in spring (21%).

INTRODUCTION

Foals are born in every month of the year, which implies that the mare is polyoestrous and capable of reproducing the year round[1]. The difficulty of getting all mares to settle during the winter months, however, supports the classic statement of Heape[2], made as early as 1900, that the mare is a polyoestrous animal with a tendency to the monoestrous state.

The mechanisms which control reproductive function have long been the object of speculation. Livestock breeders have credited the appearance of green grass, sunshine and warm weather as initiating sexual activity, but, since this sets in long before these factors make their appearance, the mechanism must be different in the mare. Since the wild species of the equine family are reported to be monoestrous the "tendency towards monoestrum" in the modern domestic mare could be interpreted as an atavistic trait, and the polyoestrous property as be an acquired characteristic brought on by breed improvement and domestication[3]. The question arises as to how far the development towards the polyoestrous characteristic has progressed, and how firmly it has become established in the modern horse.

The only studies hitherto published on the breeding season of equines in South Africa are those carried out by Kupfer[4], who worked mainly on donkeys and a few horse mares kept under ordinary veld conditions. He concluded that the appearance of oestrum in the donkey and the horse is seasonal. Then Quinlan, Van Rensburg & Steyn[5] observed that the oestrous cycles continued throughout the year in the majority of mares of the light farm type, which were stabled at night and let out in a small paddock during the day. Some mares went into true anoestrus.

MATERIAL AND METHODS

The observations which form the basis of the present study were made at Onderstepoort from January 1964 to July 1965 on 75 mares of the light farm type.

Throughout the period under review the mares were kept on natural grazing in camps at Kaalplaas, Onderstepoort. The mares received a supplementary diet of 15 lb of lucern hay daily from the middle of August to the end of October 1964 and from April to July 1965. All mares were teased daily by active, vigorous, healthy stallions and the results recorded on special teasing charts. Ovarian changes were observed by daily rectal palpations during oestrus followed by examinations made daily, or every second or third day during the interoestral period. The number of services and the service dates were recorded. Stallions were tested regularly for fertility. A pregnancy diagnosis, as described by Van Niekerk[6], was made between the 17th and 20th day after ovulation.

The same techniques were used to compare different breeds. A Thoroughbred stud in the North Eastern Cape grassveld area and the purebred Percheron stud, at Elsenburg-Stellenbosch Agricultural College were selected for the purpose.

In the Thoroughbred stud the oestrous cycles of 39 mares were investigated during two succeeding breeding seasons from August to January.

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The length of the oestrous cycle, the oestrous period, number of services and the occurrence of ovulation were recorded.

Similarly, the oestrous cycles of 31 Percheron mares were recorded for one season.

Results

Complete data on the individual breeding cycles of 188 mares of three different breeds are summarized in Tables 1 to 4.

Table 1.—Percentage of Mares showing Oestrus.

Breed.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Light farm type	87	86	100	100	100	100	96	95	69	66	53
Thoroughbred	61	59	70	80	83	80					
Percheron	66	71	86	90							
Average	71	72	85	90	91	90	96	95	69	66	53

The average percentage of all mares showing signs of oestrus during the different months of the year is given in Table 1. Except for July, when no observations were recorded, mares came into oestrus right through the year. The percentage of the available mares which came into oestrus was fairly constant for the months October to March, with an average range of 85-95% of mares in oestrus in all three breeds under observation. The average for the Thoroughbred mares was about 10% lower than for the Percheron and 20% lower than for the light farm type mares for the same period. The percentage of mares on heat showed an appreciable decline from 95% in March to 53% in June, but increased to 71% in August, 72% in September and 85% in October. This pattern of increase in the incidence of oestrus from August to December is the same in all three breeds, but in the case of light farm type a higher percentage of mares came into oestrus each month.

The data in Table 2 show a marked difference in the percentage of mares that ovulated during the different months of the year. During the period November to February, 96-100% of the mares in oestrus ovulated. Little variation between breeds was observed in this period. From March to June the average percentage of ovulations declined monthly. During June and August only 20% and 19% respectively of the oestrous periods ended in ovulation, followed by a marked increase every subsequent month to reach a peak of 100% in January.

The average conception rate of mares served during oestrus remained fairly constant throughout the year, with the exception of 41% in August (Table 3). The average conception rate for all three breeds ranged between 63 to 83%. In the mares of the light farm type the average conception rate ranged from 66% in June to 100% in October, November, December and Ja-

Table 2.—Percentage of Mares in oestrus that ovulated.

Breed	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Light farm type	25	46	92	100	100	100	97	86	81	60	20
Thoroughbred	14	44	60	97	98	100					
Percheron	20	66	92	90							
Average	19	52	81	96	99	100	97	86	81	60	20

Table 3.—Percentage of Served Mares that conceived.

Breed	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Light farm type	75	81	100	100	100	100	70	63	80	66	66
Thoroughbred		13	47	50	52	70					
Percheron	9	40	55	50							
Average	41	67	67	67	76	85	70	63	80	66	66

nuary. The lower conception figures in the Thoroughbreds and Percherons early in the breeding season were mainly due to a higher percentage of anovulatory oestrous periods in these two breeds.

TABLE 4.—PERCENTAGE OF FERTILE SERVICES.

Breed	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Light farm type	14	55	24	100	100	71	36	22	26	28	40
Thoroughbred		5	20	33	46	41					
Percheron	9	20	33	20							
Average	11	26	26	51	73	51	36	22	26	28	40

The highest average percentage of fertile services in all three breeds was obtained during November (51%), December (73%) and January (51%), with an appreciable decline to 11% in August. In June only three mares were served, of which two became pregnant (Table 3). As the number of mares served is rather small, the average percentage of 40% for June may not be a true indication. The percentage of fertile services in mares of the light farm type were the highest, mainly because of the lower incidence of anovulatory oestrous periods early in the season and perhaps more intensive veterinary supervision.

From August to November gradual monthly increases in the number of mares on heat from 71 to 90% were observed. Quinlan's[5] figures do not show a gradual increase but rather a marked fluctuation between different months from August to December. The results obtained by Kupfer[4] on 2-year-old maiden mares are of little value as most 2-year-old mares give variable results on teasing.

Seasonal variations in Follicular Development and Ovulation:

During winter and early spring a large percentage of mares, especially those with long oestrous periods of 20-30 days and longer, showed very little follicular development. In other mares again, follicular growth took place but the follicles failed to ovulate and eventually underwent atresia. Eighty-five per cent of the oestrous periods in May, June and August were of this type (Table 2). During September 48% of anovulatory oestrous periods were still observed. A marked increase in the percentage of follicles that ovulated were observed from September (52%) to October (81%). Between November and February more than 96% of oestrous periods ended in ovulation with a peak of 99 and 100% during December and January. The periods above coincide with those months when the highest percentage of mares were on heat. These observations on the breeding cycles of mares in South Africa are the only ones accompanied by data recorded after regular rectal examinations of the ovaries throughout the year. The conclusions drawn by Quinlan *et al.*[5] that none of 50% oestrous periods, occurring during April, May, June and July were accompanied by ovulation, were not confirmed by ovarian examination and are therefore of little value.

As shown in Table 2, ovulation took place in 81% of mares in oestrus during April, 60% in May and 20% in June.

Berliner[1] states that foals are born in every month of the year. During the course of my investigations Thoroughbred mare No. 5650 was served once on the 25th June, 1964, ovulated the next day (nearly the shortest day of the year), conceived and foaled on the 28th of May, 1965. In April, May and June, 80%, 66% and 66% respectively, of all mares served became pregnant (Table 3). Quinlan *et al.*[5] found that no mares, mated during the months April to July, conceived.

Sutton[7] reported that 66 per cent of mares running with a rig in a veld camp at Kaalplaas, Onderstepoort, conceived in May and June.

Most investigators found that conception rates decrease during the winter months. Caslick[8] and Berliner[1] cited instances where fertility was low during winter and early spring, although mares were bred several times throughout these long oestrous periods. During March and April conception resulted from only 15% of heat periods at the Mississippi experimental station. Observations made in Europe by Day[9], Achnelt[10] and Plas[10] and Burckhardt[11], also show that during the early part of the breeding season, i.e. February to March, breeding efficiency tends to be significantly lower than duirng the summer months May to July. This study (Tables 3 and 4) indicates that the percentage of fertile services is much lower in the winter and the early spring than in the summer, but the conception rates of served mares remain fairly constant throughout the year, especially in mares of the light farm type (Table 3). Conclusive evidence is submitted that the fertility of the ovum is not affected by the time of the year during which ovulation

occurs. The conception rates of mares that ovulate remain fairly constant throughout the year provided the mare is served near the end of the oestrous period and within 24 hours of ovulation. The lengthy oestrous periods during this time of the year and the fact that the follicle sometimes reaches its maximum size as long as 5 days before ovulation, make it difficult to estimate the most probable day of ovulation. For these reasons the percentage of fertile services during winter and early spring is much lower (Table 4).

Discussion

The most significant feature revealed by study of the data presented in Tables 1-4 is that the occurrence of oestrus in mares maintained under natural grazing conditions with supplementary feeding during the winter months, was not confined to certain months of the summer. In the majority of mares oestrus was observed throughout the year. These findings support the classic statement of Heape[2]. Asdell[12] and Day[9] state that certain mares experience a continuous series of oestrous cycles throughout the year, while others entered a period of anoestrum. Contrary to my findings, Kupfer[4] found that in South Africa the occurrence of oestrus in mares maintained under veld grazing conditions, was of a seasonal nature. He stated that ovulation and oestrus only took place during the ovulation and oestrous season, which embraces the months October, November, December, January and probably also February and March. The only other published work on oestrous cycles in South Africa (Quinlan *et al.*[5]) agrees with my finding that certain mares have uninterrupted oestrous cycles throughout the year.

The percentage of mares which came into oestrous (Tables 1-3) confirms that the mare is polyoestrous but achieves a high level of reproductional efficiency during a more restricted period of the year. From October to March over 80% of mares of all three breeds came into oestrus with a peak of over 90% in November, December and January for all three breeds and 100% in mares of the light farm type. There was an appreciable decline from 95% in March to 69% in April, 66% in May and 53% in June. This marked decline in the occurrence of oestrus between March and April was also observed by Quinlan *et al.*[5], but their figures show an increase in the occurrence of oestrus from 63.6% in April to 81.7% in June.

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PATTERN OF THE OESTROUS CYCLE OF MARES.

II. The duration of the oestrous cycle and oestrous period.

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SUMMARY

Data on the length of 360 oestrous cycles and 471 oestrous periods of 199 mares of three different breeds are summarised, analysed and discussed.

Two distinct periods can be recognised according to the average length of the oestrous cycle, namely one in summer extending from November to February with an average cycle length of 19.3 days and another in winter from May to August with cycles of 29.7 days. During spring the cycle length decreased from 28.7 in August to 21.2 days in October with an average duration of 25.5 days for this period. Combined average length of the oestrous cycle is 23.7 days and the average duration of oestrous 10.5 days, throughout the year. During early spring an average length of 13.7 days for the oestrous period was recorded. For the period November to February the length of the oestrous period in all 3 breeds averaged 5.2 days.

Irregularities in the length of the oestrous period, one of the main problems in horse breeding, were most pronounced during the months March to October and varied from 3 to 38 days. For the months December to February a variation of only 2-12 days, with an average of 4.9 days, was recorded.

INTRODUCTION

Irregularity of the oestrous period and cycle such as deep anoestrum with inactive ovaries, long oestrous periods with active but non-cycling ovaries or long oestrous periods eventually culminating in ovulation, is one of the main problems in horse breeding. It is chiefly responsible for the poor conception rate in September and October. As breeders are keen to get as many mares in foal as possible during this period, mares with long oestrous periods are served several times. This practice results in a decline in the fertility of the stallion and increases the risk of contracting genital infections.

1. *Duration of the oestrous (breeding) cycle*:

According to Asdell[1] the average length of the oestrous, or breeding cycle is 22 days, with a mean range from 19-23 days. A tabulation by Andrew and McKenzie[2] of data reported by investigators from many parts of the world shows a range of 7-124 days, a comparatively wide range in cycle length occurring even during the normal breeding season between April and July (summer). In light mares (Grade Thoroughbreds) 63% of cycles fell between 17-24 days, but long cycles of 29 days made up 10% of the total. Among the draft mares the modal length was essentially the same, but a fair number showed a peculiar tendency to have very short cycles of 10-16 days.

By establishing the interval elapsing between ovulation in successive heat periods, the same authors found that the duration of the interovulatory interval, varied over a wide range from 12-58 days in light mares and 15-41 days in draft mares, there was thus a considerable deviation from the mean of 20.6 and 20.7 days respectively. Cummings[3] established comparable norms, the average duration of the interovulatory period being 22 days with a range of 12-29 days. Hancock[4] obtained an average of 21 days with a range from 15-24 days. Quinlan *et al.*[5] found the greatest variations in stabled animals in South Africa both as regards the range and length of the cycle during the February-July period. The average length of the oestrous cycle for these six months was 35.9 days as against 23.8 days for the August-January period. Only 64.2% of the oestrous cycles fell within the normal limits of 16-25 days.

According to the literature, whichever way one measures the breeding cycle length, an average of 21 to 22 days is observed during the normal breeding season.

2. *Duration of the oestrous period*:

The oestrous period is very variable in length and, therefore, is the main contributory factor to the variation in length of the entire cycles. It is about twice as variable as the interval between the oestrous (heat) periods, according to Cum-

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mings[3]. In his data the average duration of oestrous was 5 days and dioestrous 15.9 days.

Asdell[11] concluded that the average duration of the period is between 4 and 9 days, but added, "a great deal depends upon the method of testing for heat, and upon the statistical analysis of data". Published data reviewed by Andrew and McKenzie[2] show a wider range for oestrous, viz. 1 to 103 days. Their own data show a range from 1 to 37 days with a mean of 5.3 days; 74% of oestrus periods ranged between 2 and 8 days. Light mares were inclined to have shorter periods, 40% of the periods being 3 days or less in length. In draught mares, the modal length was 5 days but the frequency distribution curves, although showing a peak at 5 days, revealed longer oestrous periods (up to 11 days) occurring frequently enough to be of practical concern to the breeder. Trum[6] in a study of over 1,500 cycles in Thoroughbred mares found that 61% of heat periods were 4-6 days long; 11%, 2-3 days; 28%, 7-9 days and only 5% over 10 days. Hancock[4] reported an average duration of 5.3 days in Thoroughbred mares.

Kupfer[7], Constantinescu and Mauch[8] and Caslick[9] have reported oestrous periods lasting a single day on the other hand the latter author observed mares to be in continuous heat for as long as 103 days. Van Rensburg and Van Heerden[10] recorded a prolonged heat period of 171 days which ended in spontaneous ovulation. Day[11] gives the average length of oestrus as varying from 3-54 days but averaging from 7-8 days.

Quinlan *et al.*[5] recorded an average duration of oestrous in stabled mares throughout the year of 6.93 days with a range of 1-55 days. Only 29.5% of the oestrous periods fell within 5-7 days, whereas 33.2% lasted 2-4 days. Oestrous tended to be longer in the early part of the season.

Belonje[12] published data on a Thoroughbred stud in the Karoo area of South Africa. He found the oestrous period to last from 1-33 days with an average of 5.0 days during the normal breeding season. Trum[6] also reported a seasonal variation in the duration of oestrous. He found a definite trend towards shorter heat periods in Thoroughbred mares from spring to midsummer. Periods of less than 4 days' duration made up 44% of the periods in March, as against 78% in July (summer).

The object of this study is to determine and compare the pattern of the oestrous cycle in mares of different breeds in the Republic of South Africa.

Material and Methods

The observations which form the basis of the present study were made on 75 mares of the light farm type, 93 Thoroughbred and 31 Percheron mares.

The mares of the light farm type were kept on natural grazing in camps at Kaalplaas, Onderstepoort. They received a supplementary diet of 15 lb. of lucerne hay daily from the middle of August to end of October in 1964 and from April to July 1965.

The Thoroughbred mares were kept on natural grass veld in the north eastern Cape and only mares in foal received supplementary feeding of lucerne hay and oats from May to September. The Percheron stud was kept at Elsenburg-Stellenbosch Agricultural College. Mares in foal were stabled at night and received a supplementary diet of lucerne hay.

All mares were teased daily by active, vigorous, healthy stallions and the results recorded on teasing charts.

Ovarian changes were observed by daily rectal palpation but in some cases this was done only every second or third day during the interoestrous period.

Results

1. *The oestrous cycle*:

Data on the length of 360 oestrous cycles of the three breeds under observation are summarised in Tables 1-7 for the individual breeds.

The average duration of the oestrous cycle of mares of the light farm type was found to vary from 18.2 days in December to 30.3 days in June (Table 1). Two distinct periods can be recognised from the average length of the oestrous cycle, namely, one extending from November to Feb-

Table 1.—Summary of breeding cycles.
Oestrous cycles of mares of the light farm type, Onderstepoort, 1964 & 1965.

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Number of mares	16	13	10	8	11	8	26	21	16	18	17
Total number of days	341	302	165	156	199	150	313	460	294	271	273
Number of cycles	12	11	8	8	11	8	16	21	12	9	9
Average length of cycle in days	28.4	27.4	20.6	19.5	18.2	18.7	19.5	21.9	24.5	30.1	30.3
Range in days	20–42	14–50	15–25	15–23	14–21	16–21	16–24	18–26	11–30	22–45	24–35

ruary, with an average length of 19.0 days, the shortest average cycle of 18.2 days being recorded during December. The second recognisable period extended from May (30.1 days) to August (28.4 days) with a mean duration of 29.6 days for this period.

The variations which occurred in the length of the individual oestrous cycles are shown by the range in days in the last line of Table 1. A marked difference between seasons exists with a range of 39 days between the shortest and longest individual cycle during the April to September period when cycles range from 11 to 50 days in length. The range for October to March was considerably less namely 12 days. During January the length of all the individual oestrous cycles falls between 16 and 21 days.

The oestrous cycles of 93 Thoroughbred mares i.e. maiden, barren and foaling mares are summarised in Tables 2-4.

Very little difference between the average length of the breeding cycle of Thoroughbred mares and mares of the light farm type was observed during the months September to January (Tables 4 and 1). A difference of less than one day in the length of the breeding cycle was observed for the months September, November, December and January and 1.5 days for October. A noticeable difference was obtained between maiden and barren mares as against foaling mares (Tables 2 and 3). In foaling mares the oestrous cycle was shorter early in the spring. The average length of the cycle for the period October to January was 18.4 days in foaling mares and 22.7 days in dry mares.

The range in the length of the individual oestrous cycle in all Thoroughbred mares varied from 14-21 (7 days) in January, to 23-38 (15 days) in September. In barren and maiden mares a difference of only 4 days was observed in January.

Tables 5, 6 and 7 show the summarised results of the individual breeding cycles of 31 Percheron mares. The total average length of the oestrous cycle for maiden, barren and foaling mares was more or less the same as in the previous two breeds under discussion for the months August and September, but 2-3 days longer during October and November. The average length in spring (August, September and October) was 25.7 days for Percheron mares (Table 7), 25.5 days

TABLE 2.—SUMMARY OF DATA OF BREEDING CYCLES.
THOROUGHBREDS: MAIDEN AND BARREN MARES, 1957 & 1958.

	Sept.	Oct.	Nov.	Dec.	Jan.
Number of mares	53	52	46	40	11
Total number of days	680	657	796	472	116
Number of cycles	24	29	38	23	6
Average length of cycle in days	28.3	22.5	20.9	20.5	19.3
Range in days	23–38	11–32	15–32	17–23	17–21

TABLE 3.—SUMMARY OF DATA OF BREEDING CYCLES.
THOROUGHBREDS: FOALING MARES, 1957 & 1958.

	Oct.	Nov.	Dec.	Jan.
Number of mares	18	29	19	6
Total number of days	277	454	340	95
Number of cycles	14	27	18	5
Average length of cycle in days	19.0	16.9	18.9	19.0
Range in days	12–36	10–27	10–24	14–21

TABLE 4.—SUMMARY OF DATA OF BREEDING CYCLES.
THOROUGHBREDS: BARREN, MAIDEN AND FOALING MARES.

	Sept.	Oct.	Nov. . .	Dec.	Jan.
Number of mares (total)	53	70	75	59	17
Total number of days	680	934	1250	813	211
Number of cycles	24	43	65	41	11
Average length of cycle in days	28.3	22.0	19.0	19.0	19.0
Range in days	23–38	11–36	10–32	10–24	14–2

TABLE 5.—SUMMARY OF DATA OF BREEDING CYCLE.
PERCHERONS: MAIDEN AND BARREN MARES, 1963.

	Aug.	Sept.	Oct.	Nov.
Number of mares	15	18	15	10
Total number of days	142	382	249	184
Number of cycles	5	13	10	8
Average length of cycle in days	29.0	29.3	24.9	23.0
Range in days	24–33	20–45	9–42	20–28

TABLE 6.—SUMMARY OF DATA OF BREEDING CYCLES.
PERCHERONS: FOALING MARES, 1963.

	Sept.	Oct.	Nov.
Number of mares	11	10	4
Total number of days	114	104	65
Number of cycles	6	6	3
Average length of cycle in days	19.6	17.3	21.6
Range in days	13–27	13–26	20–26

TABLE 7.—SUMMARY OF DATA OF BREEDING CYCLES.
PERCHERONS: BARREN AND MAIDEN AND FOALING MARES, 1963.

	Aug.	Sept.	Oct.	Nov.
Number of mares (total)	15	29	25	14
Total number of days	142	496	353	249
Number of cycles	5	19	16	11
Average length of cycle in days	29.0	26.1	22.1	22.6
Range in days	24–33	13–45	9–42	20–28

for mares of the light farm type (Table 1) and 25.2 days for Thoroughbred mares (Table 4). Again, as in Thoroughbred mares, there was a marked difference in duration of the oestrous cycle between foaling and dry mares. The foaling mares showed an average of 19.5 days for the period September to November whereas the dry mares showed 25.5 days for the same period.

The range between the shortest and the longest oestrous periods for each month was greater in dry mares (22 days) than in foaling mares (10 days) with a range of 17 days in both groups of Percherons for the months August to November. The range for mares of the light farm type was 18 days for the same period (Table 1) and 19 days in Thoroughbred mares (Table 4).

2. *Oestrous periods*:

Complete data on the duration of 471 individual oestrous periods of 199 mares are summarised in Tables 8 to 14 and graphs 1 and 2.

Table 8 is a summary of the data on the oestrous periods of 64 mares of the light farm type. The average length of the oestrous period, as well as the range for every month, is given in days. In the last line of this table the total number of mares in anoestrum is shown.

TABLE 8.—SUMMARY OF RESULTS OF OESTROUS PERIODS.
OESTROUS PERIODS OF MARES (LIGHT FARM TYPE), ONDERSTEPOORT, 1964–1965.

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Number of mares	16	13	10	8	11	8	27	21	16	16	15
Total days	269	115	130	34	112	42	165	153	112	193	187
Number of oestrous periods	14	11	12	8	19	8	30	22	12	10	10
Average length in days	19.2	10.4	10.6	5.9	5.7	5.1	5.1	6.9	9.3	19.3	18.7
Range	7–35	5–30	4–27	3–9	4–9	3–8	3–9	3–18	6–17	11–27	11–34
Total number of mares in anoestrus	2	2	0	0	0	0	1	1	5	6	6

The mean duration of oestrus varied from 5.2 days in January to 19.2 days in August. A tendency towards a seasonal pattern in the length of the oestrous period, as in the oestrous cycle, was observed. The length of the period was very constant during the months of November to February, namely 5.7, 5.7, 5.1 and 5.1 days respectively with an average duration of 5.4 days for this summer period. A gradual increase in the length of oestrus was observed from March (6.9 days) to April (9.3 days). Between April and May the length of the oestrous period increased rapidly from 9.3 days to 19.3 days. During the winter months, May to August, the length of oestrus remained more or less constant with an average of 19.1 days. The length of the oestrous period decreased sharply between August and September from 19.2 days to 10.4 and each month a gradual decrease was observed during spring, September to November.

The range between the shortest and longest oestrous period was 5 days for the summer months, November to February, with an average of 23 days for the months May to September. The shortest oestrous period, 3 days, was recorded during the summer months while the longest of 35 days in the winter and the early spring.

All non-pregnant mares of the breed under discussion showed signs of oestrus during October, November, December and January. The percentage of anoestrous cases was 4% during February and 5% in March. During April, May and June the percentage of mares in anoestrous increased rapidly to 40% of all non-pregnant mares under observation.

TABLE 9—SUMMARY OF DATA OF OESTROUS PERIODS.

THOROUGHBREDS: MAIDEN AND BARREN MARES, 1957 & 1958.

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Number of mares	24	53	52	47	36	11
Total days	278	479	510	280	125	25
Number of oestrous periods	14	32	42	43	27	6
Average length in days	19.8	15.0	12.2	6.5	4.7	4.0
Range in days	8–31	4–33	5–31	4–18	3–5	2–6
Total number in anoestrus	10	23	14	11	9	5

TABLE 10.—SUMMARY OF DATA OF OESTROUS PERIODS.

THOROUGHBREDS: FOALING MARES, 1957–1958.

	Sept.	Oct.	Nov.	Dec.	Jan.
Number of mares	10	19	30	20	6
Total days	24	105	132	89	24
Number of oestrous periods	4	16	27	19	6
Average length in days	6.0	6.5	4.9	4.6	4.0
Range in days	3–10	2–21	2–9	2–11	3–6
Total number in anoestrus	6	4	4	4	0

TABLE 11.—SUMMARY OF DATA OF OESTROUS PERIODS.

THOROUGHBREDS: BARREN, MAIDEN AND FOALING MARES.

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Number of mares (total)	24	64	71	77	56	17
Total days	278	503	615	412	214	49
Number of oestrous periods	14	36	58	70	46	12
Average length in days	19.8	14.0	10.7	5.8	4.6	4.0
Range in days	8–31	3–33	3–31	2–18	2–11	2–6
Total number in anoestrus	10	29	18	15	13	5

In Tables 9, 10 and 11 the data on 236 individual oestrous periods of Thoroughbred mares, are summarised.

In maiden and barren Thorougbred mares a noticeable difference in the length of the oestrous period was observed between the beginning and the end of the breeding season. In foaling mares this difference was not so pronounced.

In the early spring (August to October) the oestrous periods of dry mares showed an average length of 16 days ranging from 19.8 days in August to 12.2 days in October. In foaling mares the average duration for the same months was 6 days. Little difference in the duration of oestrus between the two groups of Thoroughbred mares was observed during the summer months November and January. The average mean duration for these months was 4.8 days for both groups. The difference in the length of the oestrous period during the summer months between Thoroughbred mares (4.8 days) and mares of the light farm type (5 days) is very small. The mean duration of oestrus in both groups of Thoroughbreds (Table 11) was 14 days for the period from August to October as against 13 days in mares of the light farm type for the same season. The variation which occurred in length of the individual oestrous periods is shown by the range for every month in Tables 9, 10 and 11. The smallest difference (4 days) was obtained in January when a range of 2-6 days was observed (Table 11). During summer, November to January, a range of 9 days between the shortest and the longest individual oestrous periods was observed as against an average of 27 days during spring (August to October). The average for the last period was 12 days in the group of foaling mares and 28 days in barren and maiden mares.

The total percentage of Thoroughbred mares under observation, which showed anoestrum, decreased from 41% in August to 23% in December (Table 11).

The occurrence of anoestrum during summer was higher in the group of maiden and barren mares than in foaling mares.

TABLE 12.—SUMMARY OF DATA OF OESTROUS PERIODS.
PERCHERONS: MAIDEN AND BARREN MARES, 1963.

	Aug.	Sept.	Oct.	Nov.
Number of mares	15	18	15	10
Total days	207	232	125	67
Number of oestrous periods	10	15	13	9
Average length in days	20.7	15 4	9.6	7.4
Ranges in days	24–33	8–38	5–29	3–12
Total number in anoestrus	5	5	2	1

TABLE 13.—SUMMARY OF DATA OF OESTROUS PERIODS.
PERCHERONS: FOALING MARES, 1963.

	Aug.	Sept.	Oct.	Nov.
Number of mares	4	11	10	4
Total days	17	45	83	22
Number of oestrous periods	3	7	10	3
Average length in days	5.6	6.4	8.3	7.3
Range in days	4–8	4–8	3–22	3–5
Total number in anoestrus	1	4	0	0

TABLE 14.—SUMMARY OF DATA OF OESTROUS PERIODS.
PERCHERONS: BARREN, MAIDEN AND FOALING MARES, 1963.

	Aug.	Sept.	Oct.	Nov.
Number of mares (total)	19	29	25	14
Total days	224	277	208	89
Number of oestrous periods	13	22	23	12
Average length in days	17 2	12.3	9 0	7.4
Range in days	4–33	4–38	3–29	3–12
Total number in anoestrus	6	9	2	1

In Tables 12 and 13 the individual data, with regard to the oestrous periods of purebred Percheron mares, are summarised. The combined results of both barren, maiden and foaling mares are given in Table 14.

As in the previous two breeds under observation the average length of the oestrous period decreased between August (20.7 days) and September (15.4 days) in the group of dry mares. In the combined group this period decreased from an average duration of 17.2 days in August to 12.3 in September. After September a gradual decrease each month to an average of 7.4 days in November was recorded. In foaling mares of the Percheron type as well as in Thoroughbreds, the average duration of oestrus remained more or less the same from August to November with an average duration of 7 days for the 4 months. In barren and maiden mares an average of 15 days, for the same period, was observed with an average length of 11.7 days in the combined group. The combined oestrous periods for both groups of Percheron mares for the months August to October averaged 13 days in length in comparison with an average length of 14 days in Thoroughbreds and 13 days in mares of the light farm type for the spring season.

Each month during the spring months the range between the longest and shortest individual oestrous periods became smaller. An average of 27 days was recorded for the months of August, September and October, with 9 days in November (Table 14). Again, as in Thoroughbreds, the range in length of the oestrous period in foaling mares showed less variation than that of maiden and barren mares. The difference in the individual periods averaged 9 days in foaling mares and 27 days in maiden and barren mares for the 3 months August to October. Figures for the same period for Thoroughbred mares were 9 days for foaling mares and 27 days for maiden, dry and foaling mares during these 3 months. In mares of the light farm type an average range of 26 days was observed between the shortest and the longest period during spring (Table 8).

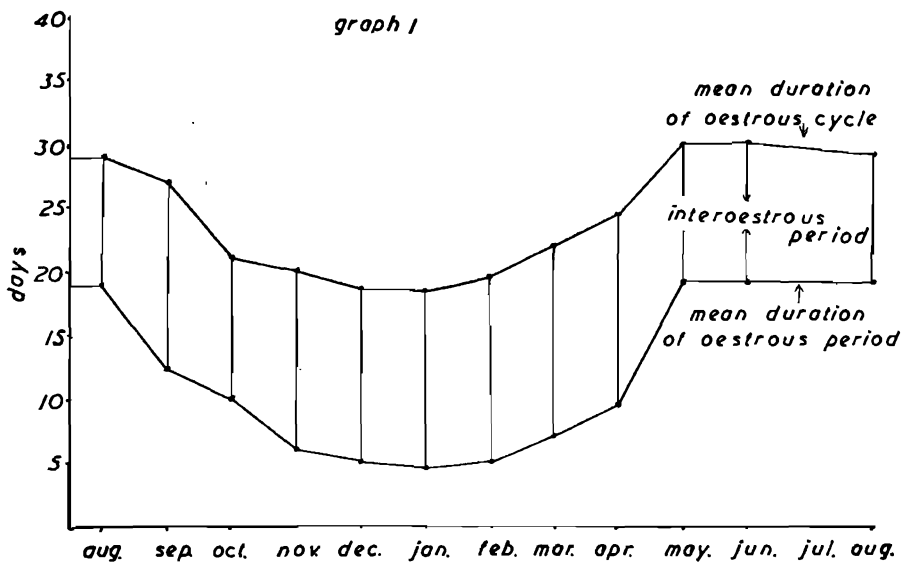
During the months of August and September 31% of all the Percheron mares under observation were in anoestrum as against 7% in November (Table 14). During August and September the percentage of foaling and barren mares, not showing oestrus was more or less the same, but in October and November all the foaling mares came into oestrus as against 88% of barren mares. 12% of the dry mares were in anoestrum.

Combined data on the oestrous cycle and the oestrous period of all 3 breeds under observation are summarised in Graph 1.

The average length of the oestrous cycle is 23.7 days and the average duration of oestrus 10.5 days throughout the year.

During spring (August, September and October) an average of 25.7 days for the length of the oestrous cycle and 13.7 days for the oestrous period was recorded.

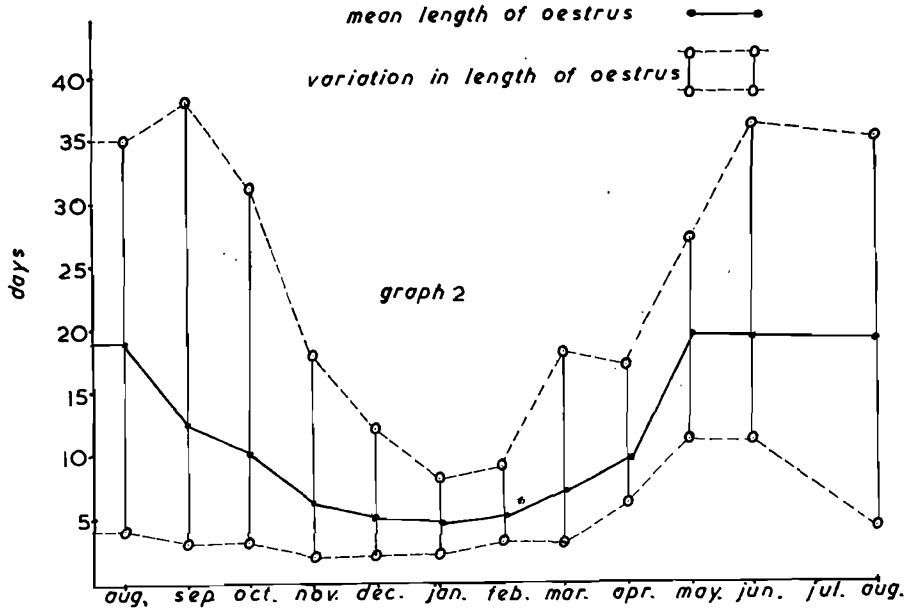
For the period November to February the length of the oestrous cycle averaged 19.3 days and that of the oestrous period 5.2 days in all three breeds. The longest average oestrous cycles were observed



from May to August, namely an average length of 29.7 days. The average length of the oestrous period was 18.8 days during the same period.

The length of the oestrous cycle is mainly governed by the length of the oestrous period. The interoestral period remains fairly constant throughout the year. There is a slight tendency for this period to become a little shorter in winter than in summer (Graph 1).

Very little difference between the average length of oestrous cycles was found in the three breeds examined (Tables 1, 4 and 7). Oestrous cycles averaged 25.7 days in spring, August to October. The shortest cycle observed for this period was 9 days and the longest 50 days. During the normal breeding season from September to February, an average length of 20.9 days was recorded in a summary of the breeding cycles of all three breeds.



The seasonal variations in the range between the longest and shortest oestrous periods for every month are illustrated. Irregularities occur in this period throughout the year. It is apparent, however, that these variations were more pronounced in winter and early spring.

Discussion

1. *The oestrous cycle*:

On a well-managed stud-farm where there is veterinary supervision, and where the management of stallions and mating of mares are given great care, the main cause of infertility is due to irregularities of the individual oestrous cycles.

In mares which were in anoestrum during winter there is a tendency towards long oestrous periods of 10-35 days in early spring (August and September). As the length of the oestrous cycle is mainly governed by the length of the oestrous period, long oestrous cycles are more prevalent early in the season.

A total average of 23.7 days was recorded throughout the year. Similar values for the inter-ovulatory period have been obtained in other parts of the world. Andrews and McKenzie[2] found that the modal length of the oestrous cycle in draft mares was essentially the same as in light breeds. They found that the largest percentage of cycles fell between 17 and 24 days with an average length of 20.6 days. Cummings[3] reported an average of 22 days and a range of 12 to 29 days, and Hancock[4] an average of 21 days with a range of from 15 to 24 days.

Investigators who measured the oestrous cycle from the first day of oestrus found the cycle to be the same length. Constantinescu and Mauch[8] reported that the average length of the oestrous cycle of "warm blood" breeds is between 19 and 24 days. Asdell[1] found an average length of 22 days with a mean of 10 to 23 days. In South Africa Quinlan *et al.*[5] observed cycles that varied in length from 6 to 113 days with an average length of 27 days during the normal breeding season. They recorded an average length of 29.9

days, throughout the year. My findings of 20.9 days for the breeding season and 23.7 days throughout the year, differ from these findings. Belonje[12] averaged 18.2 days for the interoestral period and 5.9 days for the oestrous period in the course of his investigations on Thoroughbred mares in South Africa. By excluding the findings of Quinlan *et al.*[5] an average of 21 to 23 days is obtained regardless of the manner of measuring the cycle length.

2. *The oestrous period*:

The oestrous period is very variable in length and is the main contributing factor to variations in length of the entire cycle. A range in length from 2-38 days and an average duration of 10.5 days throughout the year, in all the breeds examined, is recorded. The average duration of oestrus during the normal breeding season was 7.3 days, with an average of 5.2 days from November to the end of February. In spring (August to October) 13.7 days was recorded as the average length of oestrus.

The average length of the oestrous period for the lighter breeds, Thoroughbreds 6.8 days, and for mares of the light farm type 6.9 days is about the same, but for draft mares an average of 9.1 days was recorded during the breeding season. Andrew and McKenzie[2] found just the opposite, namely an average of 5.5 days in light mares and 5.2 days in draft mares with an average of 5.3 days for both breeds during summer. My average was 5.2 days for the same period. Belonje[12] recorded an average period of 5.9 days in Thoroughbreds and Quinlan *et al.*[5] 6.6 days in mares of the light farm type during the normal breeding season in South Africa.

Nishikawa[13] reported an average of 6.18 days in Korean ponies, Trum[6] of the U.S.A. reported that 61% of oestrous periods were from 4-6 days long in well-fed Thoroughbred mares. Day[11] found that in the majority of Welsh ponies examined oestrus lasted from 7 to 8 days and Hammond[14] recorded a period of 7 days. He also reported the average length of the oestrous period to be 11 days throughout the year. This finding corresponds with the period of 10.5 days found during my investigation but differs from that of Quinlan *et al.*[5] of 6.93 days for the whole year.

A seasonal variation in the duration of oestrus is noticeable (Tables 8-14). Long periods, averaging 18.8 days, were recorded during the winter months of May to August. A marked increase in the length of the period from April to May (9.3 to 19.3 days) and a marked decrease in the length of the period from August to September (18.7 to 12.2 days) were recorded. The shortest periods were observed during the summer from December to February with an average duration of 4.5 days in January. The range between the shortest and longest period was also much less during this period, which makes it easier to "fix" the day of ovulation during this time of the year (Graph 2). All mares under observation ovulated within twenty four hours from the end of the oestrous period irrespective of the length of this period.

The incidence of anoestrum was found to be greater during the winter and the early spring. Forty-eight per cent of mares were in anoestrum during August and September but only 16% failed to show signs of oestrus in the summer months of November to February. In mares of the light farm type these figures were very much lower. Only 7% were in anoestrum in early spring and all mares of the light farm type came into oestrus during the summer months. The mares of the light farm type received supplementary feeding in spring and the Thoroughbred mares, barren and maiden, were on natural veld grazing and in fairly poor condition. This underlines the fact that a high level of nutrition is essential for the normal breeding performance.

The problems facing the veterinarian early in the season are the occurrence of anoestrum, prolonged anovulatory and ovulatory oestrus periods and the wide variations in the length of these periods.

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A NOTE ON INTRACEREBRAL *CYSTICERCUS BOVIS* IN A CALF.

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SUMMARY

A case of cerebral cysticercosis is reported in a calf following heavy experimental infestation with 1,500,000 ova of *T. saginata*. Skeletal muscle contained a mean of 127.5 cysticerci/100 g. (Heavier degrees of intramuscular infestation occur naturally). Histopathological examination revealed little host reaction. The role of cysticerci in eliciting nervous symptoms is discussed.

INTRODUCTION

Cysticercosis of the brain and spinal cord due to *Cysticercus cellulosae* is a common neurological disorder in humans due to auto infestation. Heavily infested pigs are frequently found to harbour numerous *C. cellulosae* in the brain, and the same cysticercus also occurs in the brain of dogs. *C. dromedarii* occurs in the brain of camels and *C. ovis* has been encountered in the cerebrum of goats. Innes and Saunders[1] refer to *C. bovis* in the bovine brain without giving details or references. In the course of experimental infestation of calves, a search was therefore made for cysts in the brains of heavily infested animals.

METHODS MATERIALS AND RESULTS

Ova of *Taenia saginata* Goeze, 1782 had been administered to five calves, one to three days after birth, in doses varying from 300,000 to 1.5 x 10^6. Examination of the calves at slaughter two to four months later showed the infestation with cysticerci to vary from heavy to very heavy. Cysticerci were encountered in muscle and all visceral organs and tissues, particularly lung, heart, liver, spleen and kidneys.

Examination of the jaw muscles and the flexor muscles of the shoulder of the calves revealed from 2.2 to 127.5 cysts/100 g of muscle. The brains were closely examined by serial incision. Only in the calf showing the severest infestation were cysticerci found: two cysticerci were embedded in the cerebrum, both protruding slightly onto the surface.

Formalin fixed cerebral tissue blocks containing cysts were embedded in paraffin wax, cut at three microns and stained by the haematoxylin-eosin, Giemsa and Von Gieson methods as well as for iron.

Histological examination revealed one cyst immediately below the pia mater and extending into grey matter, and a second situated somewhat deeper. During processing the walls of the parasitic cysts became separated from the host tissue. Both cavities in the brain substance left by the cysts measured circa 3 mm in diameter, although not completely round.

The cysts had provoked very little tissue reaction on the part of the host. One was completely surrounded by a very thin collagenous capsule which varied between one and three cells in thickness. In this capsule and immediately without it, a very mild infiltration of cells comprised of a few scattered macrophages and eosinophiles, was present. A few of the larger blood vessels in the vicinity showed mild round cell cuffing. The other parasitic cyst was surrounded by a narrow reactionary zone which consisted mainly of macrophages arranged in layers which varied between one to three cells in thickness. No definite collagenous fibrils could be identified in this reactionary zone. A few scattered eosinophiles, plasma cells and lymphocytes had infiltrated into and beneath the layers of macrophages, while in two places small aggregations of round cells which appeared to consist mainly of plasma cells, occurred. Some blood vessels in the immediate vicinity showed a mild round cell cuffing, but around one blood vessel in particular the Virchow-Robin space showed a relatively severe infiltration of cells, many of which were haemosiderin-laden macrophages.

The cysts in the muscle were unarmed and fully viable *in vitro*. No nervous symptoms were seen in the calf during life.

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Discussion

Cysticercosis of the human brain due to *C. cellulosae,* is frequently reported[2,3,4,5]. Incidental cases are detected on radiological examination[4,6], but have also been implicated as the cause of epilepsy[7] and of sudden hydrocephalus[8]. Cysticerci of the chord has been reported as the cause of paraplegia in man[9,10]. Ocular cysticercosis causes retinal detachment[11] and extensive impairment of vision[12] in man. Cysticerci have been reported in the eyes of cattle[13]. In dogs, cerebral cysticercosis[14] occurs and may also lead to symptoms resembling rabies[1].

The presence of even a single cysticercus in a vital situation e.g. the *cisterna cerebromedullaris* is significant[16], but frequently cysticerci in the C.N.S. may go clinically unnoticed. The advent of subsequent degenerative changes in and around the parasite may however elicit symptoms[7]. Though frequently heavily infested, pigs are short lived, and this factor may mitigate against frequent occurrence of nervous symptoms due to cysticercosis of the central nervous system, the latter being fairly common. In South Africa, the incidence of bovine cysticercosis is recorded as varying from 3.031%[17] to 3.97%[18] of animals slaughtered, but the incidence at certain abattoirs is as high as 9.6%[18]. Urquhart[19] has recorded the longevity of *C. bovis* as 21 months. The author's own observations have shown that cysticerci in bovine muscle may remain viable for at least 36 months. It is accepted that the situation of the parasitic cyst will affect its longevity, cysts in the liver and heart showing degenerative changes within a few months. By far the majority of bovine infestations are slight and the probability of cerebral cysticercosis is accordingly of a low order. Natural infestations leading to 352 cysticerci per 100 g of muscle have however been encountered at the Pretoria abattoir. Minnings, quoted by Heinz *et al*[20], states that in man, intracerebral cysticerci require four times as long to calcify as those in muscle, the latter calcifying within 5 years. This statement has been considered to solicit confirmation[20]. Under extensive conditions, or in the case of dairy and breeding stock, heavily infested cattle may well become old enough to permit of degeneration of cysts in central nervous tissue and the eventual manifestation of nervous symptoms.

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THE CHEMICAL PATHOLOGY OF OVINE ICTERIC STATES.

1: Mechanical obstruction of the common bile duct.

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SUMMARY

The chemical pathology of common bile duct obstruction in the sheep has been studied in eleven experimentally created cases. Apart from increasing red cell fragility, no erythrocyte or leukocyte dyscrasias are present in uncomplicated cases. Hyperbilirubinaemia appears soon after bile duct occlusion, high plasma bilirubin levels are rapidly attained and bilirubinuria appears when plasma levels of 0.9-3.5 mg% of bilirubin glucuronides are evident. Photosensitivity appears after a time lag of two to five days. The reasons for this are discussed. Coproporphyrinuria is a feature of the disease. Its renal excretion is compared with that of phylloerythrin. No disturbances of kidney function are present in uncomplicated cases, but bilirubinuria and bileaciduria are accompanied by the passage of urine of low specific gravity, in the absence of frank polyuria.

An absolute rise in the plasma $\alpha 2$-globulin fraction is characteristic and is associated with increased plasma ceruloplasmin levels. Hypercupraemia occurs late in the disease and is not associated with hypercupriuria.

Plasma iron levels drop to very low values immediately after the operation, returning slowly to normal as the syndrome develops. Disturbances of plasma electrolyte balance are seen throughout the experimental disease but are interpreted as being general manifestations of stress.

The levels of activity of various enzymes in plasma are discussed and indications are given as to the value of some of these in the laboratory diagnosis of the condition. With some exceptions the chemical pathology of the experimentally produced ovine disease is essentially the same as that of the naturally occurring syndrome in man.

INTRODUCTION

The work reported in this paper formed part of the general research programme into the pathogenesis of the ovine disease known locally as geeldikkop. This disease is one in which there is severe photosensitivity and icterus, the latter being best classed as an intrahepatic cholestasis[1]. Most, if not all, of the constituents of bile appear to be returned to the blood circulation of affected animals at the height of the disease, without there being any obvious mechanical obstruction of the biliary tree[1,2,3]. Dyes like bromsulphalein, normally excreted via the bile, also fail to reach the biliary tract at this time and a biochemical lesion altering the permeability of the hepatic cell wall to various biliary constituents has been postulated as being the essential disturbance in the disease[2,3,4,5]. In order to interpret much of the chemical pathology of geeldikkop it was essential to study that of outwardly similar icteric states in the sheep. This paper reports the chemical pathology of common bile duct obstruction in the sheep. Subsequent papers in this series will deal with intrahepatic cholestatic icterus and haemolytic states.

MATERIALS AND METHODS:

(a) Experimental animals:

The sheep used in these studies were eleven adult (full mouthed) merino wethers weighing between 50 and 65 lbs after shearing, and designated numbers 1626, 1432, 1782, 2759, 2900, 18681, 19769, 21489, 21490 and 22021. The common bile duct was severed between double ligatures under chloral hydrate anaesthesia, proximal to the junction with the pancreatic duct, and the cystic duct was ligated at the gallbladder end, isolating this structure completely. The animals were kept in galvanised iron metabolism cages of standard patterns, prior to and after the operation. When urine was collected for studies on the 24 hourly excretion of porphyrins and copper, the urine collecting bottles described elsewhere were strapped to the sheep[6]. Faeces were collected throughout the experiments in standard collection bags. The sheep were fed on fresh green lucerne, crushed maize and water *ad libitum* throughout the entire experimental period. Animals were

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usually kept in the metabolism cages on this ration for at least one week prior to the operation, during which time samples of blood and excreta were taken daily to establish base-line values. The post-operation period generally lasted from 7-21 days before the sheep died or were slaughtered.

The sheep were tested for the appearance of photosensitivity by shearing and depilating their backs, heads and ears and then exposing them at regular intervals to solar radiation. Photosensitive animals showed evidence of hyperaesthesia, pruritis, pain and erythema of the depilated areas within minutes of exposure to sunlight. These symptoms were followed rapidly by oedema of the exposed parts. The exposure in each instance was limited to sufficient time for unequivocal symptoms to develop — usually 5-15 minutes in most instances.

TABLE 1.—METHODS USED.

DETERMINATION	*REFERENCE*
Red cell fragility	Brown[2]
Bilirubin	Malloy and Evelyn[39]
Plasma phylloerythrin	Perrin[40]
Plasma total cholesterol	King and Wootton[41]
Plasma alkaline phosphatase	King and Wootton[41] (AAP. method)
Plasma glutamic oxalacetic transaminase	King[42]
Plasma glutamic pyruvic transaminase	King[42]
Plasma lactic dehydrogenase	Wróblewski and La Due[43]
Plasma isocitric dehydrogenase	Taylor and Friedmann[44]
Plasma aldolase	Sibley and Lehninger[45]
Plasma phospho-hexose isomerase	Bodansky[46]
Blood urea	Brown[47]
Plasma creatinine	Folin and Wu[48]
Plasma uric acid	Caraway[49]
Plasma amino acids	Hawk, Oser and Summerson[50]
Blood sugar	Lehman and Silk[51]
Total plasma proteins	Weichselbaum[52]
Differential plasma protein analysis	van Zyl[10]
Plasma sodium	King and Wootton[41]
Plasma potassium	King and Wootton[41]
Plasma calcium	Ferro and Ham[53]
Plasma magnesium	Neill and Neely[54]
Plasma chloride	Schales and Schales[55]
Plasma bicarbonate	van Slyke, Stillman and Cullen[56]
Plasma inorganic phosphate	King and Wootton[41]
Total plasma copper	Brown[8]
Erythrocyte copper	Brown[8]
Direct-reacting copper	Brown[8]
Indirect-reacting copper	Brown[8]
Ceruloplasmin	Houchin[57]
Plasma iron	King and Wootton[41]
Urine copper	Brown[8]
Urine coproporphyrin	Rimington[58]
Urine phylloerythrin	Perrin[40]
Colloidal Gold Test	Gray[59]
Thymol turbidity and flocculation tests	McLagan[60,61]
Zinc sulphate turbidity test	Kunkel[62]

TABLE 2.—SHEEP 19769 — HAEMATOLOGICAL STUDIES.

Determination	Day 1 (Before Operation)	Day 2 (Before Operation)	Day 3 (Before Operation)	Day 1 (After Operation)	Day 3 (After Operation)	Day 8 (After Operation)
Red Cell Count (millions/cu. mm.)	7.3	7.4	7.2	7.2	7.1	7.0
White Cell Count (thousands/cu. mm.)	14.7	12.8	14.2	15.6	10.6	9.5
Packed Cell Volume. %	33	32	32	31	31	32
Haemoglobin g%	7.21	7.31	7.01	7.12	6.87	7.62
Erythrocyte fragility (% in 0.7% NaCl)	20.1	19.0	20.2	20.0	22.9	29.7

(b) Analytical Methods:

Haematological studies were made using standard techniques as also were the qualitative urine analyses. The methods used for all other chemical studies on blood and urine are listed in Table 1. Photometric measurements were made either in a Unicam SP.500 spectrophotometer or in an Evans Electroselenium (E.E.L.) portable colorimeter. Flame photometry of sodium and potassium was performed on the E.E.L. model A instrument.

All reagents used were "Analytical Reagent" grade. Enzymes and substrates used for the enzymatic methods were obtained from the Sigma Chemical Co. (St. Louis, Mo.) and were of the purest grades offered by this firm.

Results

(a) *Haematological studies*:

The data obtained from sheep 19769 and reproduced in Table 2 are typical of these from all the eleven sheep in most respects. No changes of note were found in the values for packed cell volume, red cell count or haemoglobin in any of the animals. With one exception the total white cell counts fell gradually as the experiment progressed in the manner shown in Table 2. The exception was sheep 1782, which developed peritonitis. The white cell count rose in this animal from 7.5×10^3 to 31.52×10^3 before the case terminated fatally. In all but one animal there was a steady increase in red cell fragility. In the exception (sheep 18681), the value for this determination remained unaltered. In all the other animals the increase in fragility was more pronounced than that seen in sheep 19769; increases being in the order of two to three times the pre-operation values.

(b) *Bile pigment and bile acid excretion*:

Hyperbilirubinaemia develops rapidly following common bile duct obstruction in the sheep, and values for total plasma bilirubin in the order of 10-20 mg% may be found 5-8 days after total obstruction. The data from sheep 2900, presented in Table 3, are typical.

Cases of this nature generally terminate fatally within three weeks of establishment of the obstruction and are the rule in experiments of this type. Three out of the eleven of our cases developed a collateral bile excretion system within a week to ten days of the operation. This phenomenon, which was noticed by Quin[7] in his earlier work on biliary function in sheep, was confirmed at autopsy after slaughter of all three cases. The data from sheep 1432, presented in Table 3, are typical of the plasma bile pigment levels in these cases.

Table 3.—Plasma Bile Pigment Levels

(All values are expressed as mg%)

	Days before Operation					Days After Operation												
	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11	12	13
Sheep 2900:																		
Total Bilirubin	0	0	0	0	0	0.36	1.43	2.86	6.79	13.21	13.93	14.29	14.64	16.43	17.85	16.07	18.57	20.05
Bilirubin Glucuronides	0	0	0	0	0	0.36	1.07	1.43	4.29	7.89	9.29	8.93	10.00	11.79	12.74	11.07	11.42	15.55
Bilirubin	0	0	0	0	0	0	0.36	1.43	2.50	5.32	4.64	5.36	4.64	4.64	5.11	5.00	7.15	5.50
Sheep 1432:																		
Total Bilirubin	0	0	0	0	0	4.29	4.29	10.71	16.07	19.29	20.71	19.29	19.29	7.14	4.29	0.72	0.51	0.20
Bilirubin Glucuronides	0	0	0	0	0	2.86	3.22	6.07	10.72	12.65	13.21	12.55	13.21	4.29	2.07	0.41	0.30	0.10
Bilirubin	0	0	0	0	0	1.43	1.07	4.64	5.35	6.64	7.50	6.74	6.08	2.83	2.22	0.31	0.21	0.10

Clinical icterus is first discernible two to three days after the operation at total bilirubin levels of 2.5-3 mg% and rapidly increases in intensity in cases which terminate fatally.

Bilirubinuria appears one to two days after closure of the common bile duct at plasma bilirubin glucuronide levels of 0.9-3.5 mg%, and its increasing severity parallels that of the hyperbilirubinaemia. Typical data on which these statements are based are given in Table 4.

Urobilinogen generally disappears from the urine two to four days after bile flow into the intestines has ceased (see also the relevant data in Table 4). In animals where a collateral biliary excretion pathway has become re-established, the time of re-appearance of the pigment in the urine is very variable and seems to depend largely on the degree of gastro-intestinal motility in the animal concerned.

Urobilinogen levels in the faeces generally started to decline about two to three days after bile duct obstruction and was generally absent

TABLE 4.—QUALITATIVE EXAMINATION OF THE URINE OF CASES OF COMPLETE COMMON BILE OBSTRUCTION FOR BILE PIGMENTS AND BILE SALTS — COMPARISON WITH THE PLASMA LEVELS OF BILIRUBIN GLUCURONIDES.

Sheep No.	Bilirubin glucuronide in plasma (mg%)	Bilirubin glucuronide in urine	Urinary Urobilinogen	Urinary bile acids
1626: 28–8–56	0	0	+	+
29–8–56	0	0	+	+
30–8–56	0	0	+	+
Operated				
4–9–56	0	0	+	+
5–9–56	0.5	0	+	2+
6–9–56	3.5	+	±	2+
7–9–56	4.0	3+	0	3+
8–9–56	6.25	3+	0	3+
10–9–56	6.30	3+	0	4+
2759: 7–10–56	0	0	+	+
8–10–56	0	0	+	±
9–10–56	0	0	+	±
10–10–56	0	0	+	+
Operated				
11–10–56	0	0	+	+
12–10–56	0.9	±	+	+
13–10–56	1.2	+	+	2+
14–10–56	2.8	+	±	2+
15–10–56	3.8	2+	0	3+
16–10–56	4.4	4+	0	3+
17–10–56	4.2	4+	0	3+
18–10–56	4.4	3+	0	3+
19–10–56	4.2	4+	0	3+
20–10–56	4.3	4+	0	3+
21–10–56	4.2	4+	0	3+
2900: 16–11–56	0	0	+	+
23–11–56	0	0	+	+
26–11–56	0	0	+	+
27–11–56	0	0	+	+
28–11–56	0	0	+	+
Operated				
4–12–56	0	0	+	2+
6–12–56	0.50	0	+	+
7–12–56	1.07	+	±	2+
9–12–56	2.21	2+	0	2+
12–12–56	5.43	3+	0	3+
13–12–56	7.86	5+	0	4+
15–12–56	9.29	5+	0	4+
16–12–56	8.93	5+	0	5+
17–12–56	10.00	5+	0	5+
18–12–56	11.79	4+	0	5+
19–12–56	12.14	5+	0	5+
20–12–56	11.08	5+	0	5+
21–12–56	12.55	5+	0	5+

from the faeces in most cases about six to seven days after the operation.

Plasma bile acid levels were not determined in this particular investigation but the urine from all the animals was tested qualitatively for these compounds. Typical results of such tests are given in Table 4. A marked increase in the urinary excretion of bile acids occurs within one to three days after bile duct obstruction. Bileaciduria is then severe and sustained until the animal dies, unless collateral excretory ducts are re-established, when it then decreases rapidly in severity.

(c) *Porphyrin excretion*:

The relevant data is presented in Table 5. The first date given in each case is the date immediately following the operation. In all six animals the base-line values for plasma phylloerythrin were less than 1 mcg%. Once the bile ducts are obstructed, plasma levels of this porphyrin rise steadily *but not dramatically* in cases which do not develop collateral bile ducts. When considering these figures it must be borne in mind that the operation is preceded by a 24 hour starvation period and is usually followed by gut stasis which may last for one to three days. Photosensitization appears from three to five days after the biliary obstruction is established, *i.e.* at plasma levels of the porphyrin above 5.0 mcg%.

Phylloerythrin is undetectable in the urine of normal sheep by the method used. Obstruction of the bile duct is followed by an immediate appearance of this porphyrin in the urine in variable amounts.

Urinary coproporphyrin excretion in all six animals varied between 0-14 mcg/24hrs. Common bile duct occlusion is followed by immediate and *often dramatic* increases in the levels of urinary excretion of this pigment. These levels exceed in general the levels of phylloerythrin output in the urine.

Sheep 18681 is typical of animals in which collateral bile flow is reconstituted. Plasma levels of phylloerythrin may fall rapidly once this happens and photosensitivity disappears. The urinary excretion of both porphyrins under discussion likewise decreases rapidly.

TABLE 5.—PLASMA AND URINE PORPHYRINS.

Sheep No. and Dates	Plasma Phylloerythrin mcg%	Urine Phylloerythrin mcg/24 hrs	Urine Coproporphyrin mcg/24 hrs
21490: 20–9–66	0.9	7.7	110.9
22–9–66	5.1	39.9	234.5
26–9–66	5.3	12.6	182.6
27–9–66	6.9	24.4	132.6
21488: 20–9–66	1.9	29.8	43.8
22–9–66	6.3	83.6	86.31
26–9–66	5.6	16.6	13.6
27–9–66	8.3	10.9	105.2
21489: 21–9–66	2.4	18.5	35.9
23–9–66	2.7	26.8	23.1
27–9–66	5.3	21.3	51.8
22021: 21–9–66	4.3	10.9	18.2
23–9–66	5.3	54.9	170.9
27–9–66	9.5	25.1	71.1
*18681: 21–9–66	2.4	18.3	38.9
23–9–66	1.6	10.3	22.2
27–9–66	1.4	4.2	7.7
19769: 21–9–66	3.7	2.3	3.1
23–9–66	4.8	39.2	105.8
27–9–66	6.5	47.1	155.4

*This sheep developed colateral bile excreting ducts.

(d) *Liver function tests*:

The use of the colloidal gold flocculation, thymol turbidity, thymol flocculation and zinc sulphate turbidity tests in the diagnosis of ovine liver disease are discussed elsewhere[8]. These tests must be performed on the day of collection of the particular blood sample. Under such conditions the colloidal gold flocculation test gives negative results in uncomplicated ovine obstructive icterus; values lower than 5.0 are obtained for the thymol turbidity test and lower than 5.0 for

the zinc sulphate turbidity test, while negative or at the best slightly positive results are found with the thymol turbidity test. Storage of plasma or serum for longer than 48 hours in the refrigerator gives rise to false positive reactions with the *flocculation tests and abnormally high turbidity* readings.

Total plasma cholesterol values show a steady and sustained rise after common bile duct occlusion except when collateral excretion has become re-established (e.g. sheep 18681). The values presented in Table 6 illustrate this point. The first date in each case noted in this table is, as before, the day immediately after the operation. No base-line values were higher than 167 mg%.

TABLE 6.—PLASMA TOTAL CHOLESTEROL.

Sheep No.	Date	mg%
21490	20–9–66	163.6
	22–9–66	254.6
	26–9–66	484.9
21488	20–9–66	163.6
	22–9–66	254.6
	26–9–66	230.3
21489	21–9–66	151.5
	23–9–66	242.4
	27–9–66	266.4
22021	21–9–66	157.6
	23–9–66	242.4
	27–9–66	297.0
18681	21–9–66	121.3
	23–9–66	175.8
	27–9–66	100.0
19769	21–9–66	127.3
	23–9–66	193.9
	27–9–66	242.4

Values obtained for plasma alkaline phosphatase in our experimental cases are represented by the figures given in Table 7 for sheep 2900 and 1432. The variations in the plasma levels of this enzyme before and after operation are such that no particular conclusion can be drawn from these figures.

Plasma levels of activity found for glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactic dehydrogenase (LDH), isocitric dehydrogenase (ICD), aldolase (ald) and phosphohexose isomerase (PHI) following common bile duct occlusion are presented in Table 8. Units given here are as defined in the original *procedures. Pre-operation values for these determinations* were well within the normal limits determined by Wagner and Brown[9] in all cases, namely, GOT, 41-212; GPT, 7-87.5; LDH, 393-896; ICD, 65-950; Ald, 0-14.7; PHI, 47-117. Units are as defined earlier[9].

The figures found after bile duct occlusion are interesting and important as regards their interpretation. The only figures falling throughout within the normal ranges are those for GPT and ICD. The general trends for GOT, LDH, Ald and PHI are high values immediately after the operation, returning slowly to normal levels over the next week irrespective of the nature and outcome of the case. These data suggest that the elevations in the activity of these enzymes in plasma are as a result of the extensive tissue trauma which is part of the operation and not a result of common bile duct occlusion.

(e) *Kidney function*:

Urea, creatinine, uric acid and total amino acids were determined on blood samples from all the animals taken every second or third day throughout the experiments. No significant alterations of the blood levels of these nitrogenous compounds were observed in any of the sheep during the post-operation period of two to three weeks.

Qualitative urine examinations were carried out on samples collected every day throughout the entire experimental period. The tests included those for specific gravity, pH, albumin, sugar, ketones, bile pigments and blood. No changes of note other than those already mentioned earlier, and those in the 24 hourly volume of urine voided and its specific gravity were observed. Common bile duct occlusion was followed, within one to two days after operation, in every instance by a marked decrease in the specific gravity of the urine passed. This coincided in every instance with the appearance of the bilirubinuria and

TABLE 7.—PLASMA ALKALINE PHOSPHATASE
(Values are King-Armstrong Units.)

Sheep No.	Days before operation				Days after operation.										
	4	3	2	1	1	2	3	4	5	6	7	8	9	10	11
2900: Units:	8.84	10.00	17.25	7.31	8.84	4.99	8.08	7.31	9.45	18.38	12.75	50.25	20.03	20.03	39.00
Sheep No. 1432: Units:	9.38	5.08	9.72	18.84	10.00	21.54	12.7	11.92	39.00	16.50	16.50	18.38	57.60	57.60	10.88

TABLE 8.—MISCELLANEOUS PLASMA ENZYMES.
(Units are as defined in the original procedures.)

Sheep	Date	GOT	GPT	LDH	ICD	Ald	PHI
21490	20–9–66	433	85.5	1550	192	170	193
	22–9–66	305	57.4	1330	255	94	167
	26–9–66	232	34.0	1160	218	31	132
21488	20–9–66	533	48.5	2000	192	154	180
	22–9–66	348	38.5	1770	250	99	182
	26–9–66	233	29.0	930	114	31	109
21489	21–9–66	448	66.5	2700	245	129	253
	23–9–66	330	35.0	1950	230	131	243
	27–9–66	230	35.5	2140	237	25	45
22021	21–9–66	365	29.5	1700	242	129	253
	23–9–66	348	12.0	1670	250	43	239
	27–9–66	225	32.0	1240	254	19	59
18681	21–9–66	225	39.5	2600	252	62	209
	23–9–66	245	11.5	1640	255	34	240
	27–9–66	138	17.0	1180	153	18	32
19769	21–9–66	363	51.0	1900	264	80	217
	23–9–66	300	38.5	1300	248	49	210
	27–9–66	220	13.0	1710	260	36	50

persisted as long as this symptom was present. In four out of the eleven animals the low specific gravity was associated with obvious polyuria. In the remaining animals the volume of urine passed each day either did not alter significantly or decreased somewhat, following the operation. The figures presented in Table 9 illustrate these points.

TABLE 10.—PLASMA PROTEINS

(All values are expressed in g%. TPP = total plasma proteins; Alb = albumins; $\alpha 1$, $\alpha 2$, β and γ refer to the globulin fractions, and γ-T is the trailing fraction.[10] The first date under each sheep is the date immediately after that of operation.)

Sheep No.	Date	TPP	Alb	$\alpha 1$	$\alpha 2$	β	γ	γ-T
21490	20–9–66	8.17	3.94	0.38	1.06	0.58	1.06	1.15
	22–9–66	6.16	2.99	0.32	0.95	0.32	0.79	0.79
	26–9–66	9.37	3.84	0.70	1.69	0.64	1.16	1.34
21488	20–9–66	6.94	3.61	0.40	0.93	0.53	0.80	0.67
	22–9–66	5.80	2.91	0.31	1.02	0.23	0.86	0.47
	26–9–66	7.52	3.19	0.43	1.56	0.61	1.21	0.52
21489	21–9–66	8.55	3.46	0.67	0.96	0.77	1.63	1.06
	23–9–66	8.20	2.85	0.47	1.30	0.36	2.23	0.99
	27–9–66	9.20	3.29	0.51	1.52	0.51	2.19	1.18
22021	21–9–66	6.85	2.07	0.50	0.79	0.40	1.79	0.3
	23–9–66	7.42	3.17	0.44	1.21	0.38	1.78	0.44
	27–9–66	8.63	3.22	0.57	1.33	0.47	2.47	0.57
18681	21–9–66	6.68	2.46	0.49	0.69	0.39	1.67	0.98
	23–9–66	7.06	2.58	0.35	1.05	0.35	1.61	1.12
	27–9–66	6.25	2.16	0.54	0.97	0.54	1.29	0.75
19769	21–9–66	7.52	3.76	0.48	0.87	0.32	1.82	0.24
	23–9–66	7.85	3.75	0.45	1.28	0.26	1.79	0.32
	27–9–66	8.38	3.58	0.60	1.50	0.50	1.80	0.40
Normal 80% Range[10]		6.85—8.23	3.66—4.91	0.16—0.51	0.52—0.87	0.27—0.61	1.02—1.96	0.04—0.56

TABLE 9.—TWENTY-FOUR HOURLY URINE VOLUMES AND URINE SPECIFIC GRAVITY.

Sheep No.	Days before operation									Days after operation.																
	4		3		2		1			1		2		3		4		5		6		7				
	Vol	SG	Vol	SG	Vol	SG	Vol	SG		Vol	SG	Vol	SG	Vol	SG	Vol	SG	Vol	SG	Vol	SG	Vol	SG			
1626	620	1.060	520	1.045	350	1.055	240	1.050		240	1.020	305	1.015	1440	1.005	1440	1.010	1160	1.010	1040	1.010	1080	1.005			
2759	2580	1.020	1660	1.020	1400	1.025	760	1.040		520	1.015	380	1.015	400	1.015	620	1.015	320	1.015	460	1.015	520	1.010			

(f) *Adrenal function and electrolyte balance*:

Water and electrolyte balance and adrenal function in general were assessed from the results of the following determinations: blood sugar, total plasma proteins, albumins and globulin fractions, plasma sodium, potassium, calcium, magnesium, chloride, bicarbonate and phosphate and absolute eosinophile counts.

Blood sugar values showed no deviations from the accepted normal range in any of the eleven animals, which could be considered as pathological. Before bile duct occlusion values ranging between 44-66 mg% were encountered. The range after operation in all eleven animals was 30-71 mg%.

Total plasma protein values before ligation of the common bile duct and the values for the various protein fractions, obtained from all eleven animals, fell within the normal 80% ranges established by Van Zyl[10]. These ranges are indicated in Table 10. The relevant data obtained from six of the sheep after operation is presented in the same table.

In all instances except those sheep in which collateral bile flow developed (e.g. sheep 18681 in Table 10), a steady rise in the values for total plasma proteins was apparent as the experiment proceeded. A study of the haematological data given earlier will show that this rise was not associated with a corresponding increase in red cell count, packed cell volume or haemoglobin. It was therefore not due to haemoconcentration. The figures in Table 10 indicate that the rise in total plasma protein levels is an absolute one due to marked increase in the α-2 globulin fraction. In some instances slight elevations of α-1 and γ-globulins also occurred. Such elevations were not consistent. The albumin : globulin ratio for sheep plasma proteins has been established as being 0.82-1.76[10]. Before closure of the bile duct no deviations from this range where seen in any of the sheep used. Following the operation ratios in the range 0.52-0.81 were shown by sheep 21489, 22021 and 19769 throughout the post-operation period. The remaining three animals showed a decrease in the A : G ratio from 0.93-1.08 just after operation, to 0.69-0.74 at the termination of the experiment.

Electrolyte balance studies were done on sheep 21490, 21488, 21489, 22021, 18681 and 19769. Plasma anions and cations were approximately balanced throughout the duration of the experiment in sheep 21489, 22021 and 19769. In the remaining sheep the total anions fell to generally about 5-10 meq/L below the values for total cations once biliary obstruction had become fully established. The lowered values were due to de-

creases in the bicarbonate ion (mean value of 28.0 meq/L down to e.g. 18 meq/L in one case), or the chloride ion (from a mean of 99.9 meq/L down to 90.9 meq/L in one case, and 81.8 meq in the third animal). These lowered values were associated with a fall in urine pH from a mean of 8.0 before operation, to 6.0-6.5. In the other animals where plasma electrolyte balance was maintained, urine pH varied between 7.0 and 8.5 during the post-operation period. In all six animals plasma sodium values fell slightly after operation, but in the animals where plasma ionic balance was maintained, such decreases were generally countered by slight increases in potassium and magnesium. Plasma calcium values remained constant throughout the experiment and inorganic phosphate levels fluctuated within the range 1.55-6.27 meq/L depending on the behaviour of the other anions in the six animals under consideration.

Absolute eosinophile counts were performed on all six animals mentioned above, and in all cases counts fell from pre-operation levels of 150-250/cu.mm. to 0-20/cu.mm. remaining at these low levels for the rest of the experimental periods.

(g) *Copper metabolism*:

Normal values for blood copper fractions in the merino sheep under South Africa conditions have been established by Brown and co-workers[11]. The normal 80% ranges and outer 9% limits for these fractions are given in Table 11. "Direct reacting" copper is that fraction which reacts with the colour reagent used without prior acid treatment and is copper presumably loosely bound to albumin, while the "indirect reacting" fraction is that which is tightly bound to globulin and which must first be released in an acid medium before reaction with the colour reagent[12-16].

In a previous publication[2], I reported that total plasma copper remains normal in sheep over periods varying from 7-8 days after biliary obstruction. Sheep 1626, 2759, 1432, 1782 and 2900 were used for these earlier studies. In our subsequent work involving the remaining six sheep the experiment was allowed to proceed longer. In five out of the six sheep total plasma copper rose slowly within the normal limits for this determination for most of the post-operation period, but rose sharply on the 9th or 10th day after bile duct occlusion. The figures in Table 11 are representative. This terminal sharp rise was not associated with failure of kidney function (see earlier data). The exception was sheep 18681 in which excretion of bile was restored by collateral ducts. Total plasma copper values fluctuated in this animal within the outer 9% limits of the normal ranges given in Table 11. In two out of the five animals (sheep 21488 and 19769) the steady rise in total plasma copper was associated

TABLE 11.—BLOOD COPPER FRACTIONS

Sheep No.	Date	Total Plasma Cu mcg%	Red Blood Cell Cu mcg%	"Direct Reacting" Cu mcg%	"Indirect Reacting" Cu mcg%	Caerulo-plasmin mg%
21490	19/9	120.6	198.4	0	120.6	15.4
	20/9	161.9	174.9	3.7	158.2	21.0
	22/9	181.0	206.1	4.3	176.7	19.3
	26/9	234.9	202.4	0	234.9	18.6
	29/9	460.0	127.7	0	460.0	21.0
21488	19/9	90.4	154.3	0	90.4	12.7
	20/9	115.8	134.4	6.4	109.4	13.2
	22/9	136.2	223.9	3.9	132.3	13.8
	26/9	173.0	250.0	4.84	168.16	13.6
	29/9	850.0	196.6	44.07	805.93	17.4
21489	20/9	204.7	77.1	5.6	199.1	19.9
	21/9	158.7	71.5	0	158.7	19.0
	23/9	188.8	209.5	0	188.8	15.45
	27/9	205.8	167.8	0	205.8	11.8
	29/9	700.0	121.6	1.69	698.31	14.2
22021	20/9	138.0	68.9	0	138.0	21.5
	21/9	155.5	93.5	0	155.5	19.0
	23/9	209.5	211.6	10.29	198.2	18.15
	27/9	269.2	65.6	0	269.2	15.7
	29/9	540.0	190.4	0	540.0	20.1
Normal Values	Range (80%)	97–160	17.5—118	0— 6.7	92–163	4.5—10.1
	(98%)	90–223	2.5—190	0—20.0	90–220	2.0—13.0

with a rise in the "direct reacting" albumin fraction. In all five animals the increasing copper levels were mainly associated with the tightly bound globulin fractions. Ceruloplasmin levels rose within 24 hours of bile duct occlusion in all six cases, and with the exception of sheep 18681 in which a return to pre-operation levels was soon seen, and were maintained at the generally high levels indicated in Table 11.

Red cell copper showed a steady and sustained rise above the normal limits after bile duct occlusion in two of the animals only, viz. sheep 21488 (Table 11) and 19769. Figures above the upper 9% limit of the normal range occurred throughout the experiment in the cases of sheep 21490, 21489 and 22021, without following any definite trend. The copper content of the erythrocytes of sheep 18681 rose steadily to 241 mcg% during the first three days after bile duct ligation (the figures concerned being 202, 236 and 241 mcg%) but fell during the next three days to well within the normal limits and remained there for the rest of the experiment.

The 24 hourly urinary excretion of copper was followed in seven of the eleven sheep, Base-line figures from these animals and three additional sheep used for other experiments gave a range for the urinary excretion of this element of 1.14-214.5 mcg per 24 hours and a mean value of 67.7 mcg per 24 hours. The range of excretion in the seven animals in which bile ducts were obstructed was 5.5-141.8 mcg per 24 hours with a mean value of 63.4 mcg. The range covers the entire post-operation period of 8-10 days. The terminal rise in plasma copper was not associated with increased urinary excretion of the element.

(h) *Iron metabolism*:

Plasma iron levels were studied in seven of the eleven animals used and varied between 80-130 mcg% before common bile duct occlusion. The operation was followed within 24 hours by a severe drop in plasma iron in every instance. A slow rise to the pre-operative levels occured over the following three to five days. This phenomenon is illustrated by the typical data presented in Table 12.

TABLE 12.—PLASMA IRON LEVELS.
(All values are mcg%. The first date given for each sheep is the day immediately following the operation).

Sheep No.	Date	Plasma Iron
21490	19–9–66	46·9
	20–9–66	12·2
	22–9–66	112·2
	26–9–66	93·3
	29–9–66	94·4
21488	19–9–66	55·1
	20–9–66	22·4
	22–9–66	59·2
	26–9–66	90·0
	29–9–66	111·1
18681	20–9–66	59·2
	21–9–66	36·7
	23–9–66	75·01
	27–9–66	112·5
	29–9–66	77·7

We are unable to explain this very constant feature of common bile duct obstruction in the sheep at the moment.

DISCUSSION

Increases in erythrocyte fragility have been noted during the course of some ovine diseases[4,5,17]. The figures for the slow but steady increases observed in these experimental animals never exceeded the normal limits set by Wagner[18] for this determination on clinically normal sheep from the Onderstepoort pool. Phenomena of this nature may be non-specific features of severe stress in sheep.

Hyperbilirubinaemia appears soon after bile duct obstruction and rapidly attains severe proportions. The 24 hourly excretion of bilirubin in the sheep averages 47.3 mg%[19]. It has been calculated that if this amount of pigment was returned to the systemic blood circulation of a sheep weighing about 55 lbs (the mean weight of the sheep used in this work) with an average plasma volume of 1676 ml over a period of 24 hours, then a plasma concentration of total bilirubin of 2.82 mg% would be attained[8]. If this rate of regurgitation of bilirubin glucuronide into the blood was maintained, a plasma level of 19.74 mg% could be reached in about seven days. That this can occur is shown by the figures presented in Table 3.

Bilirubinuria appears when plasma bilirubin glucuronide levels reach 0.9-3.5 mg%. The renal threshold in the sheep for this pigment must therefore lie in this range.

The pigment regurgitating into the blood after common bile duct occlusion is bilirubin glucuronide, but the figures shown in Table 3 indicate that a fair proportion of the total pigment present in the blood at any given time after the operation may be bilirubin. The glucuronide is known to be labile and lightsensitive[20,21]. It is possible that even though the determinations were done as soon after collection of the blood as possible, some hydrolysis of the conjugated pigment took place. The obvious explanations for the presence of fair amounts of bilirubin in the plasma of these animals after bile duct obstruction, are firstly, intravascular haemolysis, which can be discounted on the grounds of the haematological data presented, and hepatocellular injury. The presence of this latter condition cannot be

excluded, if the data in Table 8 are taken into account.

Some observations made during this work regarding the appearance of photosensitivity are interesting and important. In the first instance, there was a time lag in all the animals studied of two to five days between bile duct occlusion and the appearance of this symptom. This can be accounted for by the facts that the operation was always preceded by a 24 hour starvation period and was invariably followed by gut stasis which lasted for one to three days. Absorption of phylloerythrin during periods like these must be minimal. Furthermore the symptom appeared only after plasma levels of the porphyrin rose above 5 mcg%. Apart from frank polyuria or the excretion of urine of low specific gravity during the period of bilirubinuria, kidney function remained undisturbed in all animals during the experimental period of two to three weeks. Coproporphyrin appears to be rapidly cleared from the plasma of the sheep with common bile duct obstruction and is found in large amounts in the urine of these cases. Plasma levels of phylloerythrin remain on the other hand fairly high and the urinary excretion of this pigment is always less than that of coproporphyrin in spite of the large amounts of it which must be absorbed from the gut once gastro-intestinal motility has been restored. This remark applies specifically to animals fed on green lucerne *ad libitum*, as these were. On a diet such as these animals were receiving the mean 24 hourly excretion of the pigment in the bile of normal sheep is about 670 mcg while that of coproporphyrin is only about 80 mcg[19].

Goldberg and Rimington[22] have indicated that coproporphyrinogen is far more easily excreted by the kidneys than coproporphyrin and that over 95% and possibly all of the porphyrin is excreted in this form. All the work done on phylloerythrin so far seems to indicate that it is absorbed from the gut and is present in blood and bile as the porphyrin and not as the porphyrinogen[8]. By analogy with the work cited, phylloerythrin may not be cleared easily from the circulating blood by the sheep kidney and hence can build up to levels at which it produces photosensitivity fairly soon if it is being absorbed from the gut at a rapid rate.

Low urinary specific gravity in the presence of severe bilirubinuria and bileaciduria is well known in bile duct occlusion in human patients and is frequently not associated with obvious polyuria[23]. As mentioned earlier the same phenomenon is a feature of ovine bile duct obstruction as well.

Electrolyte disturbances such as noted here are a feature of non-specific stress conditions in sheep and have been noted in other icteric syndromes in this animal[2].

Bile duct obstruction in humans and dogs is accompanied by increased liver copper levels but seldom by elevations of plasma copper, even when large amounts of copper are given orally or intravenously to such patients over long periods[15, 16, 24]. Urinary excretion and direct excretion through the intestinal wall are increased[15, 24]. The sheep is known to have a pattern of copper metabolism different from other animals. The concentration of this element in the liver is higher in the sheep than in most other species and increases to toxic levels with even moderate increases of dietary copper, which have little effect on other species[25]. Sheep liver has the ability to complex quite large amounts of copper in a relatively stable form and to lose that excess at a very slow rate of elimination[25]. The development of characteristically high liver copper levels in the sheep is due to a lesser ability to restrict the storage of the element, rather than to the absorption of excessive amounts[26].

Bile is the main route of excretion of copper in sheep[25] and in the merino sheep on diets of green lucerne and crushed maize *ad libitum* the 24 hourly output ranges from 13-1120 mcg per 24 hour with a mean value of 133 mcg[19]. Values for the urinary excretion of copper by the sheep in the same order as those established by us in the animals before bile duct occlusion have been reported by Beck[25]. It is apparent from the data presented here that uncomplicated common bile duct obstruction in the sheep is not accompanied by any increase in urinary excretion of the element during the first two weeks of the condition.

After absorption from the gut copper is loosely bound to plasma albumin and transported rapidly to the liver, bone marrow and other organs where it is stored, becoming incorporated into cuproproteins. After a variable but short time this copper re-appears in the plasma firmly bound in the α-2 globulin fraction, associated particularly with ceruloplasmin, being incorporated into it by synthesis or exchange[12-15, 27-31]. Although most of the plasma copper is firmly bound to the α-2-globulin ceruloplasmin, the total blood copper is evenly distributed between the erythrocytes and the plasma[15]. Under some circumstances red blood cells may serve to transport copper in blood[15].

The experimental data presented earlier indicate an absolute increase in the plasma globulin and particularly in the α2-globulins following bile duct occlusion in the sheep. This increase occurs rapidly after the operation, is sustained and asso-

ciated with a similar rise in ceruloplasmin. The sequence of events following bile obstruction as regards copper metabolism in the sheep seems from the data presented, to be something like the following: free biliary copper re-attaches to the apo-cuproproteins in the liver and as these become saturated, passes out into the blood stream as ceruloplasmin; the labile plasma copper fraction may increase if most of the liver copper binding protein is saturated at the time of the obstruction; considerable amounts of copper appear to pass into the red cells as well as into the α2-globulins of plasma and the sharp terminal rise in plasma copper is probably associated with binding of copper by the other globulins, as is known to occur in human subjects[30,31]. Under these circumstances the urinary excretion of copper appears to remain constant in the sheep. In most of the animals high red cell copper levels were associated with increased red cell fragility.

The chemical pathology of common bile duct occlusion in sheep is very similar to that in the corresponding syndrome in man with respect to the plasma, urine and faecal bile pigments, bileaciduria, coproporphyrinuria, increase in total plasma cholesterol and negative reactions in the colloidal gold and thymol flocculation tests, and the thymol and zinc sulphate turbidity tests[32,33]. The elevated levels of GOT, LDH, Ald and PHI immediately after operation are not typical of common bile duct obstruction but are consequent to the widespread tissue damage which is part of the operation concerned[34-36]. This is illustrated by the figures in Table 8 which indicate a steady fall in all instances from the initial high values while the syndrome of bile duct obstruction is developing rapidly. The possibility of secondary hepatocellular injury consequent to the operation and the subsequent rise in intrahepatic biliary pressure cannot be excluded by the data presented in this paper. The persistence of high levels of LDH activity in the plasma of these cases is noteworthy. In humans and dogs, conditions involving tissue necrosis e.g. myocardial infarction, muscle trauma, necrotizing pancreatitis and fulminant haemolytic states are associated with markedly elevated plasma LDH activity[34]. Alterations in LDH activity associated with tissue necrosis depend on the amount of enzyme present in the necrotic tissue, the extent of the necrosis, the rapidity with which it occurs, the rate of loss of enzyme from the tissue, and the normal range of plasma LDH activity[34]. Markedly increased plasma levels of this enzyme have been reported in other conditions involving muscle injury in sheep, together with similar increases in GOT, Ald and creatine phosphokinase[17,37,38].

The results of this work show that laboratory diagnosis of common bile duct obstruction in the sheep can be made by taking cognisance of bile pigment levels in plasma, urine and faeces, the nature of the bile pigments in plasma, and the level of total cholesterol and the activity levels of GPT and ICD in this medium, together with the results of the flocculation and turbidity tests mentioned. Plasma alkaline phosphatase determinations are of little value in this regard, and myopathy, hepatocellular injury or other tissue damage must be excluded if estimations of plasma GOT, LDH, Ald and PHI activity levels are made.

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My professional assistants Adriana Wagner, Anna Brink and Lulu van Zyl, and my technicians P. J. de Wet, R. Gray and R. J. Briel have a pre-eminent claim to my gratitude. A glance at the contents of Table 1 will give some small indication of what they had to do. It was largely due to their readiness and unflagging zeal that we were able to make this study as comprehensive as it is. Professor Richard Clark gave his usual discerning advice and encouragement during the course of the work. The Chief, Veterinary Research, Onderstepoort, is thanked for permission to publish this paper.

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Obituary

DR. GIOVANNI MARTINAGLIA, D.V.Sc. (TORONTO), M.Sc. (CORNELL), B.V.Sc. (S.A.)

Dr. Martinaglia was the first White baby born in Roodepoort. In early years he experienced adversity and spent part of his childhood in the Langlaagte Orphanage which he regularly revisited in later life. However, as a worker tending the valves of the cyanide tanks on the Randfontein Estates gold mine, his fortunes improved when he won several hundred pounds in a sweepstake and he immediately packed his bags to study in N. America.

He had a brilliant academic career, obtaining a B.V.Sc. degree from the University of Toronto in 1919 and his M.Sc (Cornell) in 1920 for a thesis "Direct Isolation of the Human Bovine and Avian Tubercle Bacilli". During 1921 he worked under Dr. Theobald Smith at the Rockefeller Institute, Princeton, after winning a fellowship of £300. From 1921-1922 he was Demonstrator in Pathology at Johns Hopkins Medical School, Baltimore, and during this period went to Jamaica to undertake bacteriological work on leprosy.

In 1922 he returned to S. Africa and obtained a B.V.Sc. degree. He took the position of Veterinary Research Officer offered by Sir Arnold Theiler and worked on bacteriological problems concerning the preparation of mallein, tuberculin and other biological products. He distinguished himself as a research worker at Onderstepoort and in 1929 the University of Toronto awarded him the D.V.Sc. degree for a thesis entitled "Diseases of Domestic Animals in South Africa due to Organisms of the Salmonella Group".

In May 1930 Dr. Martinaglia accepted appointment as a municipal veterinarian in the Johannesburg City Council Abattoir and Livestock Market Department where he was subsequently appointed Director before retiring on pension on 11th May 1948.

He started a new career at the King George V Hospital, Durban, where he did tuberculosis research for 15 years and was held in great esteem by Dr. B. A. Dormer.

Tony Martinaglia was respected and loved by his staff. Always tolerant and friendly he had a quiet sense of humour, and was especially interested in the scientific problems which he encountered in his work. He was a keen collector of Africana, especially sketches and etchings, and had one of the four existing sketches of George Honeyball who discovered the Reef.

He died in the South Rand Hospital, Johannesburg on 10th May 1967, at the age of 79 years. To his wife, two sons and daughter we extend our deepest sympathy.

Obituary

GORDON McINTYRE.

The Association lost one of its oldest, most loyal and respected members through the death in Queenstown on 12th May 1967 of Dr. Gordon McIntyre.

Born in Lenzic, Lanarkshire on the 9th November 1886 he graduated as a M.R.C.V.S. at the Glasgow Veterinary College in May 1910, after obtaining a medal for botany and a distinction certificate.

After doing private practice in London in England for four years during which time he was also a member of the Territorial Force Veterinary Corps, he was mobilised with the 54th Division at the outbreak of World War I in 1914. He subsequently served with the 74th Division as D.A.D.V.S., with the rank of Major, and with the Southern Division in the Army of Occupation in Germany.

He came to South Africa in 1920 and joined the Division of Veterinary Services (Field) Serving as State Veterinarian successively at Vryheid, Eshowe, Frankfort, George, Kingwilliamstown, Alival North and finally at Queenstown. Though he retired in 1946 he was retained by the Division in a temporary capacity till 1958.

After he finally left the Department of Agriculture Dr. McIntyre carried on in private practice in Queenstown for another three years.

Wherever he served and whether in the State Service or in a private capacity, Dr. McIntyre won the respect, not only of stock owners, but of all others with whom he came into contact. He was known everywhere for his exemplary character, the thoroughness and conscientiousness displayed in the performance of his work, and his willingnes to serve and to help stock owners.

G. McIntyre

He was also very active in animal welfare wor and was Secretary of the S.P.C.A. in Queenstow for 18 years. In the field of sport he was a kee golfer and bowler up to the time of his death.

He leaves a wife and three daughters, two o whom are in England at present and the thir married to a farmer in Dordrecht district. T them we extend our sincere sympathy in thei great loss.

BOOK REVIEW

POST MORTEM EXAMINATION OF RUMINANTS by H. Winter. University of Queensland Press, St. Lucia, Queensland. Pp. 103 Fig. 89. Published price A $ 3.75.

This book gives a step by step account of the necropsy procedure of ruminants which has been developed by the author who is a teacher of pathology at the University of Queensland. Each step is illustrated by means of a line drawing, the sheep having been used as a model for the illustrations. The book is divided into several chapters which include: precautions against infection, instruments, external examination of the carcase, opening of the animal, removal of organs, examination of the removed organs, dissection and examination of the head and spinal cord, taking of specimens for laboratory examinations and the post mortem report.

It is intended, primarily for veterinary students and qualified veterinarians, and the use of a standard routine for necropsies is stressed not only because this facilitates the procedure but also because, with experience, one learns that there are no short cuts in performing such an operation, and no organ or part should be overlooked. The confidence of the animal's owner in the veterinarian will also be greatly enhanced by a skilfully conducted and intelligently interpreted post mortem examination. The method described has been so designed that one person can dissect a large animal without unnecessary strain and lifting of heavy organs.

While certain of the necropsy techniques differ slightly from the procedure which is taught to prospective veterinarians in South Africa, the two are essentially the same. For instance, the author prefers to open the abdomen and thorax from the right side while in this country we prefer the left approach in order, primarily, to expose and examine the spleen as soon as possible, and thus to exclude the possibility of some acute infectious disease associated with a tumour splenis at an early stage of the examination. The importance of making routine multiple incisions across the larger bile ducts in the liver for examination for liver fluke is not mentioned, although the author does state in the preface that no emphasis has been placed on any particular disease.

One of the many useful hints given in the book is the inclusion of ordinary "pruning shears" for cutting bone in place of the more expensive and, perhaps, less efficient surgical bone cutters. Two types are described, a smaller type for operation with one hand, and a larger one operated with two hands for heavier work.

The major part of the book is concerned with the actual post mortem techniques but the other chapters are of no less importance. Prevention of infection to the prosector is stressed, as is the manner in which the post mortem report is completed, the lesions described, and specimens for laboratory examination taken.

This guide to routine procedures for the post mortem examination of ruminants has admirably fulfilled its purpose and it is felt that by its publication a gap has been filed in veterinary literature. The script is written in simple language and the illustrations are easy to follow.

R.C.T.

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