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CHRYSOMYA BEZZIANA VILLENEUVE — SOME OBSERVATIONS ON ITS OCCURRENCE AND ACTIVITY IN THE EASTERN CAPE PROVINCE

J. A. F. BAKER*, W. M. MCHARDY**, J. A. THORBURN*** AND G. E. THOMPSON*

INTRODUCTION

The southerly infiltration of the screw-worm fly, *Chrysomya bezziana* Villeneuve throughout those tropical and subtropical regions of Southern Africa favouring its development is reflected, albeit sparsely, by the literature. Jack's[1] note in 1918 of its occurrence in Southern Rhodesia was the first recorded instance of the activity of this parasite below the 15th parallel. Bedford[2] cites the 1923 collection and identification of *C. bezziana* larvae in Zululand. Some economic importance was attached to the presence of the fly in the Northern Transvaal in 1934 by Jack (in Cuthbertson[3]). Zumpt[4] observed specimens in the Central Transvaal in 1940. Although Patton & Evans[5] claimed that "*Chrysomya bezziana* was common in the tissues of animals in South Africa especially in the Cape Colony", it is likely that, in this instance, loose geographical terminology led to a misrepresentation of facts and that, as suggested by Shoebotham[6] in 1947, the distribution of the screw-worm fly in Southern Africa was confined to Rhodesia, Northern, Western and Eastern Transvaal, Botswana, Mocambique, Swaziland, portion of Northern Natal and Zululand.

In 1950, however, larvae removed from infested wounds on cattle in the districts of the Eastern Cape Province by Whitehead[7] were identified as *C. bezziana*.

Some observations on the activity and behaviour of the fly in this last area are presented.

INTRODUCTION OF SCREW-WORM TO THE EASTERN CAPE

C. bezziana first made its appearance in the Eastern Cape in the Alice district in October, 1950. Shortly afterwards cases of screw-worm myiasis in cattle were reported from the neighbouring district of Fort Beaufort and from farms bordering on the Fish River Valley. Apparently favoured by the low lying and heavily bushed country of this latter area, the fly spread southwards towards the coast, strikes being encountered in the Grahamstown and Bathurst districts within a few months. By the end of April-May, *C. bezziana* activity had been recorded in that area of the coastal belt stretching from Alexandria in the east to Peddie in the west.

Little activity was evident during the winter of 1951, but, with the advent of summer, investigations revealed that cases of fly strike were occurring on the western fringe of the East London district, bordering on the Peddie area. An attempt was made by the senior author to visit as many of the properties from which such reports were received, and constant contact was maintained with large numbers of farmers throughout the East London and neighbouring districts in an attempt to plot the spread of the fly through these previously uninfested areas.

Within the ensuing nine months period a definite west to east spread of fly activity occurred, confining itself principally to the coastal belt where the well wooded and bushy valleys and plains, interspersed with numerous rivers and streams, probably constituted ideal environmental conditions for the development of the fly. This spread is substantiated by the cases investigated, each being, as far as could be determined, the first within any one area. These in chronological order were:

October, 1951 — Keiskama, Chalumna.
December, 1951 — Kidds Beach, Gulu.
January, 1952 — Fort Jackson, Igoda.
January, 1952 — East London.
February, 1952 — Gonubie, Kwelera.
March, 1952 — Cintsa, Haga Haga.
April, 1952 — Mooiplaats, Komgha.

Since this initial distribution of the fly has apparently occurred and, although occasional cases of myiasis caused by wandering flies or due to the movements of infested livestock can undoubtedly occur in adjacent areas, the present limits of srew-worm activity in the Eastern Cape would appear to be bounded by the Sundays River in the west, the Kei River in the east, and by a line running through Komgha, Kei Road, King William's

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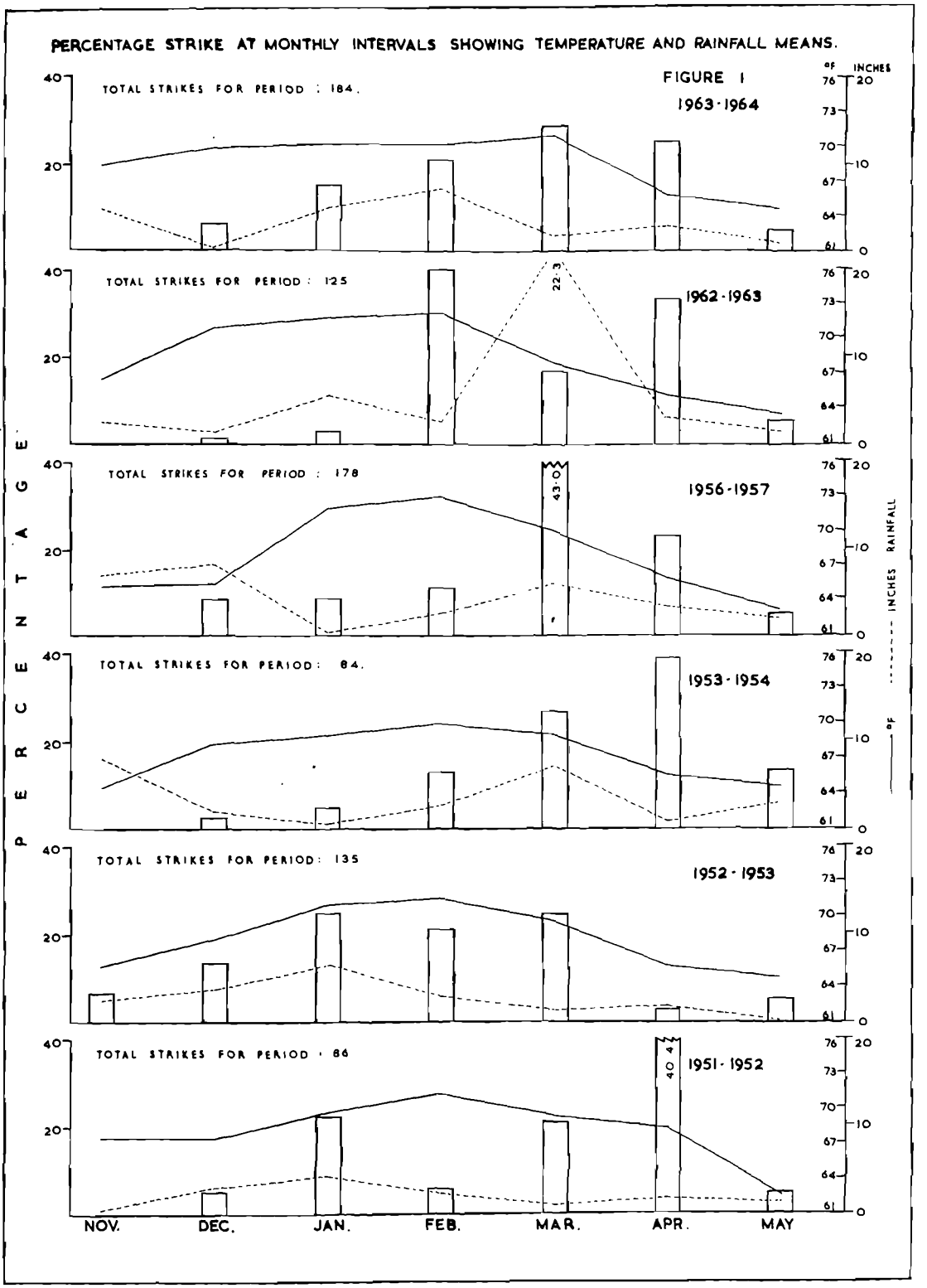


Town, Alice and Grahamstown in the north.

That such *Chrysomya* populations have become firmly established over the intervening 17 years is beyond doubt as evidenced by the constantly recurring number of cases of myiasis recorded during the summer months.

Various theories have been advanced regarding the introduction of the screw-worm fly into the Eastern Cape but it appears most probable that this was artificially assisted by the return by rail of large numbers of local cattle to this area after a period of summer grazing in the Northern Transvaal, a procedure necessiated by the crippling drought experienced in the Cape Province during the years 1949 and 1950, these animals presumably being hosts to developing larvae during their six to ten day journey.

The possibility of a natural spread down the east coast of the continent from Zululand to the Komgha district, over the 400 miles separating the previously southernmost known distribution zone of the fly, is considered unlikely in view of the west to east distribution pattern experienced in the Eastern Cape, and the complete absence of reports regarding strikes from within this intervening coastal area. This occurrence can be regarded as being the first recorded instance of the artificially induced establishment of *C. bezziana* within an area previously free of this parasite.

SEASONAL OCCURRENCE

Concurrent with the first instances of screw-worm fly strike in the coastal districts east of the Keiskama River, three farms, averaging 1,000 acres in extent, within five miles of one another in a common climatological, topographical and ecological region, were selected for research on chemical control. Approximately 250 head of cattle were grazed on each farm. Weekly visits were made to these farms, commencing with the advent of the first known cases in the early summer months and continuing throughout the remainder of the warm weather period, until a decrease in the number of strikes experienced no longer warranted further investigation. Records of the number of strikes occurring during each weekly interval were maintained. The data, presented in figure 1, are reflected as the percentage of cases for each month, together with the relevant mean rainfall and temperature readings for the district.

Although, as later discussed, screw-worm incidence is largely controlled by seasonal tick infestation, a correlation between fly activity and an increase in the mean temperatures recorded is evident, and again, although probably less significant, between rainfall and the monthly number of strikes. Commencing in November — December and reaching a peak in February — April, fly activity was dramatically reduced in May of each year concurrent with the advent of cooler weather and reduced precipitation.

Sporadic strikes however, have occurred from time to time within the district as early as September and as late as July but instances such as these reflect purely a casual occurrence rather than an established feature.

PREDISPOSING CAUSES

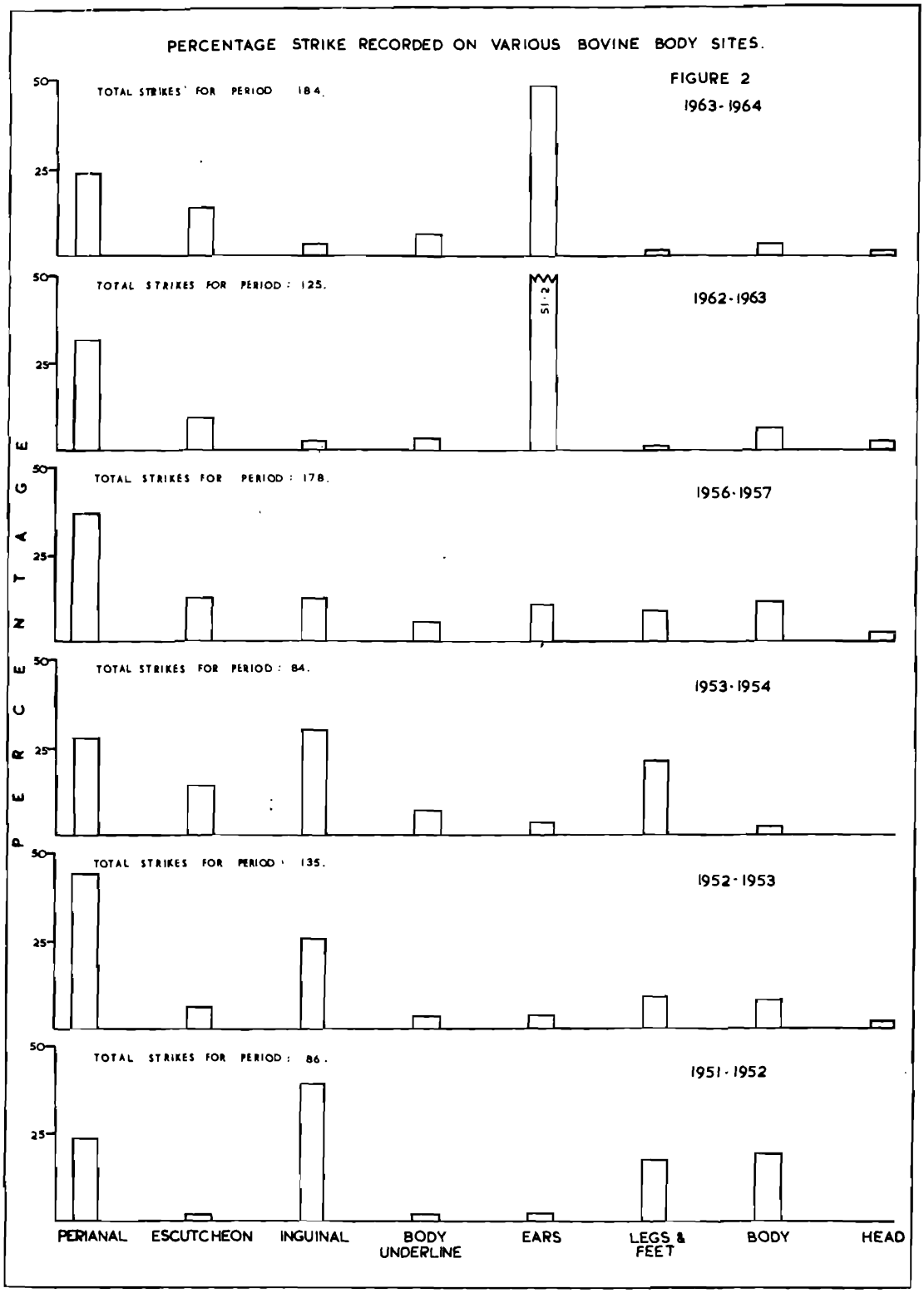
In the coastal areas of the Eastern Cape, cattle are particularly subject to attack by heavy infestations of the adult stages of various of the deep-feeding and clustering multi-host ticks, including the Bont tick, *(Amblyomma hebraeum)*, the red-legged tick, *(Rhipicephalus evertsi)*, and the Brown Ear tick, *(Rhipicephalus appendiculatus)*. Abscesses and skin damage at the sites of attachment afford admirable conditions for egg laying by the gravid female fly. As the incidence of the adult stages of these ticks also increases with the advent of the summer months, a large percentage of all strikes encountered in the Eastern Cape occurs on those parts of the body most favoured by one or more of these tick species.

By virtue of the predilection sites and less damaging feeding habits of the Blue tick, even gross infestations of this widely occurring single host tick do not predispose necessarily to screw-worm attack. In the large number of myiasis cases examined by the authors, not one instance has been suggestive of strike preceded by such infestation.

Bont-legged ticks, *Hyalomma* spp), were not encountered during investigations on the above-mentioned three farms.

Records maintained on these properties included details of each wound examined in relation to its site on the bovine body. These data, expressed on a percentage basis, are presented in figure 2. The described sites are:

1. **Perianal:** That area embracing the vulva and/or anus, including the ventral surface of the tail root, and the surrounding bare periphery.



2. **Escutcheon:** That area extending from a point a few inches below the vulva or anus to the commencement of the udder in the female, or a point a few inches posterior to the scrotum in the male.
3. **Inguinal:** The area of the groin and including the udder or scrotum and site of the rudimentary teats in the male.
4. **Body underline:** The ventral aspect of the belly anterior to the inguinal region described, including the prepuce, axillae, brisket and lower extremities of the dewlap.
5. **Ears:** The inner and outer surfaces of the ear flap and the inner ear grooves.
6. **Legs and feet:** As such.
7. **Body:** The remainder of the body surface after exclusion of the other sites described.
8. **Head:** As such, but excluding the ears.

The data must of necessity be viewed in the light of the tick infestations occurring on the animals, otherwise a completely different pattern of site choice may have emerged. Sites 1-5 (see legend, fig. 2) are favoured areas of attachment for one or more of the adult multi-host tick species referred to, predisposing them to fly strike. Sites 6-8, however, are subject to trauma as result of horn pokes, barbed wire tears, thorn punctures, foot rot, horn damage, burst abscesses or abrasions.

Correlations between the percentage of strike recorded on any one site, and various contributory factors can be made.

The perianal region is a consistently favoured site for fly strike throughout the period of observation. This area is notoriously difficult to keep free of ticks, particularly in the case of females, and at the height of the tick season will often harbour Red-legged, Bont and Brown Ear ticks. Healing of damaged skin tissue is slow on this site due to constant fouling by faecal matter. General observations in the field have shown that, even under conditions of minimal tick activity, this site is prone to screw-worm attack.

The obvious decline of strikes in the inguinal region can be attributed largely to the reduction in the Bont tick populations on the farms following the introduction, in 1951, of cattle dipping formulations highly effective against this particular tick species.

The gradual increase in the number of strikes recorded on the escutcheon can be explained partly for the years 1962-63 and 1963-64 by the clustering of Brown Ear ticks at this site, and, possibly, by a variation in interpretation of definition of sites between recorders; it cannot be attributed entirely to tick life.

The spectacular rise in the number of ear strikes occurring during the final two years of observation is related directly to the appearance in the East London district in 1962 of a strain of the Brown Ear tick highly resistant to the then most commonly employed commercial cattle dipping materials[8]. Gross infestation of the inner and outer surfaces of the ear by these ticks leads to considerable skin damage aggravated by rubbing or scratching. During peak infestation (January -March) large numbers of fresh ticks are attaching daily, giving the ear little chance to heal, with the result that this site may remain continuously attractive to the fly. Offered so readily available an egg-laying site, the female fly will tend to ignore other areas of the body. This is indicated by the detailed results (not presented in this paper) which show that in the February-March periods for 1962-3 and 1963-4 respectively, more than 80% of recorded strikes occurred on the ears. Study of figure 2 reveals that in these two seasons, the overall percentage increase reflected for ear strike for the whole of the summer period was at the general expense of all other sites, bar the perianal area and escutcheon. The habit of the Brown Ear tick in clustering elsewhere on the body, notably the escutcheon and perianal regions, when their ear predilection sites become virtually untenable due to mechanical damage, probably results in a higher than normal number of infested wounds developing on these two areas at this time.

The increase in foot strike in 1953-54 can be attributed largely to the very wet conditions which occurred in the early part of the summer season, when approximately 30" of rain fell in October-November, resulting in a relatively high incidence of foot rot, advanced cases of which were readily struck by the fly.

The close interrelation between multi-host tick worry and fly strike in the Eastern Cape is indicated by the summarised data of figure 2, presented in table 1.

Concomitant with an increase in fly activity in the summer season of 1963-64, the normally expected measure of control with dieldrin was not obtained, and it was sus-

Table 1.—STRIKES RECORDED ON VARIOUS BOVINE BODY SITES
SUMMARY OF ALL STRIKES 1951 - 1964

Favoured Sites of Multi-Host Tick Attachment					Non-Favoured Sites of Multi-Host Tick Attachment		
Perianal	Escutcheon	Inguinal	Body Underline	Ears	Legs and Feet	Body	Head
241	79	118	32	182	60	61	10
33.3%	10.1%	14.8%	4.4%	22.9%	7.8%	7.9%	1.2%
	652 83.6%					131 16.4%	

A marked recession in multi-host tick activity occurred during the years 1958-1961. This in turn gave rise to intermittent fly strike on the three experimental units under observation and continuity of inspection was abandoned for this period.

BEHAVIOUR OF *C. BEZZIANA* TO CHEMICAL CONTROL

One percent dieldrin, formulated in a grease base for topical application to screw-worm infested wounds, was employed with great success as a chemical control measure since the fly first made its appearance in the Eastern Cape. The excellent larvicidal action of this compound, coupled with its persistency in or around the wound site, effected dramatic recovery following one treatment only of all but the larger or more awkwardly situated lesions.

Treatments were generally undertaken at weekly intervals, coinciding with the regular collection of stock for dipping, except in those instances where short term inspections were desired to ascertain the larvicidal effect of new compounds. This procedure ensured that the treatments employed were all subjected to a field challenge under natural conditions.

Concomitant with an increase in fly activity in the summer season of 1963-64, the normally expected measure of control with dieldrin was not obtained, and it was suspected that some alteration in the susceptibility by the fly to this compound had developed. A comparison of the effects of treatments with dieldrin based greases undertaken during the summer periods of the years 1951-1965 are given in table 2.

The consistency with which dieldrin effected a high degree of control following one treatment only during the 1951-57 period is obvious and is in marked contrast to the figures obtained for the years 1963-65.

Collective harvests of fully developed *third instar* larvae were made during 1962-63 from untreated wounds and subjected to immersion for 60 seconds in various concentrations of dieldrin as a water miscible concentrate. After treatment the larvae were placed on filter paper for 30 seconds to remove excess liquid, thence removed to gauze covered jars half-filled with dampened vermiculite and incubated at 26°C. These results are shown in table 3.

Although no comparative figures are available, the fact that larvae survived immersion in a concentration of 10,000 ppm dieldrin can be considered of some significance.

The suspected development of a strain of screw-worm flies tolerant to dieldrin is not altogether surprising, in view of the regular employment of this compound for the treatment of larval infested wounds over the preceding twelve years.

Table 2—COMPARISON OF TREATMENTS EMPLOYED — 1951 - 1964

USING 1.0% DIELDRIN

Year	Total of Wounds Requiring One Treatment only	Total of Wounds Requiring Two Treatments only.	Total of Wounds Requiring Three or More Treatments	Percentage of Wounds Requiring One Treatment Only
1951 - 52	10	2	—	83.3%
1952 - 53	25	4	1	83.3%
1955 - 56	19	1	2	86.3%
1956 - 57	61	8	1	87.1%
1963 - 64	5	11	13	17.2%
1964 - 65	5	6	5	31.2%

Table 3—LABORATORY IMMERSION OF C. BEZZIANA

TREATMENT	Total Larvae Treated	Total of Non Pupating Larvae	Total of Larvae Pupated of Normal or Near Normal Appearance but Failing to Emerge	Total of Adults Emerged	Percent Adults Emerged
1250 ppm Dieldrin	15	3	8	4	26.6%
2500 ppm Dieldrin	23	10	9	4	17.3%
5000 ppm Dieldrin	54	29	20	5	9.2%
10000 ppm Dieldrin	54	40	12	2	3.7%
20000 ppm Dieldrin	31	30	1	0	0
Water Controls	54	4	2	48	88.8%

* 'Solvent scald' effect obvious on larvae during and after immersion.

Subsequent investigations have revealed that 2.0% fenitrothion and 4.0% dichlorfenthion effect a highly satisfactory degree of control over suspected tolerant screw-worm larvae.

BREED SUSCEPTIBILITY — CATTLE

The animals maintained on the three properties involved in this work comprised South Devon, Friesland and Shorthorn type milking herds respectively, with a large proportion of Afrikaner and Afrikaner cross-bred type male and female range cattle as well as numerous native-owned animals. Farm husbandry did not necessarily favour any one group as far as exposure to fly strike was concerned: all groups were grazed under fairly similar conditions. The primitive open kraal environment under which milking was done may have enhanced the attentions of the fly due to the daily grouping of the animals under insanitary conditions.

Under the conditions of tick infestation existing on these properties the perianal area of Afrikaner or Afrikaner cross-bred females was found to be the site most consistently

struck. This was attributed largely to the structure of this site, especially the vulva and the contiguous area, which is devoid of hair and exhibits a relatively large expanse of wrinkled skin folds, in which multi-host ticks are prone to cluster.

Apart from this one salient feature, it could not be said that any one breed or type of animal displayed more susceptibility than another to screw-worm attack; the criterion of whether an individual would be struck or not depended principally on the degree of tick infestation carried and the nature of the lesions such infestations produced.

Norris and Murray [9] have indicated the possibility of an innate myiasis resistance in cattle of Zebu ancestry. In the absence of tick challenge it would be of interest to investigate the possibility of such a mechanism in the indigenous African breeds.

HOSTS OTHER THAN CATTLE

Two cases only of screw-worm myiasis amongst domestic stock other than cattle have been recorded by the authors. One, on the perianal region of a horse, was attributed to multihost tick infestation, whilst the other, on the withers of a donkey, was located within the site of a deep wound, the cause of which was unknown.

In view of the large number of bovine strikes concurrently observed, the authors would concur with Norris & Murray [9] that "apparently *C. bezziana* is more specifically attracted towards cattle than towards other animals" as well as Zumpt's [10] observation that "the most commonly infested animals are cattle." It can be argued that, as cattle are the prime hosts of tick life in the Eastern Cape and that, as has been shown in this paper, such tick life is the chief predisposing cause of screw-worm myiasis, this is not necessarily a fair reflection. Nevertheless, equines, sheep and dogs are not infrequently subjected to fairly serious tick worry and yet are seldom attacked by *C. bezziana*, even though the fly is known to be active against cattle in the same area. It might also be supposed that myiasis due to *Lucilia cuprina* might predispose sheep to screw-worm attack but this does not seem to be the case.

Zumpt [4] in recording a case of screw-worm myiasis in a Bushbuck (*Tragelaphus scriptus* Pallas) remarks on the puzzling fact that very few occurrences of infestation in wild animals have been reported. He suggests that this must occur quite often but is not necessarily recorded.

One such case, also in a Bushbuck, was observed by the senior author in November, 1955, on one of the farms: The animal, a ram, was evidently in distress as it made little effort to retreat into the safety of the dense bush cover upon being approached, and remained standing for some minutes until a firearm had been obtained. It was eventually shot in the head from a distance of only twenty-five yards. Upon examination, a large wound, more than twelve inches in diameter and grossly infested with all larval stages of *C. bezziana* was observed on the left flank. Puncture of the rumen had taken place as was evidenced by the welling-out of stomach contents and the staining of the coat below the site. Many adult screw-worm flies were present and paid little heed to the activity of the investigators. Egg batches were scattered about the less liquified parts of the damaged tissue of the wound but it was not ascertained if these in fact were those of *C. bezziana*.

Whether the wound alone was responsible for the lethargy of the animal, could not be determined.

Two other cases of screw-worm strike in Bushbuck have been reported to the authors, one a large lesion, probably a gunshot wound, on the shoulder of a ram, again grossly infested with all larval stages, and the other on the foreleg of an ewe trapped in a wire snare in which case small numbers of larvae were present in the injured tissue. The incidents were not personally investigated but the veracity of the informants, one a local farmer and the other a nature reserve ranger, both of whom were familiar with screw-worm strike, is unquestioned.

Zumpt [4] has intimated at the probable importance of wild animals as reservoirs of infection, especially of those species which have as their favoured habitat dense riverine bush and natural forest. When it is considered that possibly many hundreds of larvae successfully completed their development in the flank wound of the Bushbuck referred to above, the significance of even sporadic screw-worm strikes on such animals should not be underestimated.

LUCILIA CUPRINA AS A SECONDARY PARASITE ON CATTLE

The rôle of *Lucilia cuprina* as a myiasis-producing agent in cattle is not unknown in

South Africa. On occasion it had been observed in the Eastern Cape by the authors before the advent of the screw-worm fly in the area. It has not been possible to implicate *L. cuprina* as a likely initiator to *C. bezziana* infestations in cattle since this latter parasite first made its appearance in the Eastern Cape, but of interest is the fact that a relatively large percentage of screw-worm wounds have been found to harbour a secondary invasion of *L. cuprina* larvae. These larvae have been observed together with *C. bezziana* larvae within the confines of the lesion, being particularly active in those parts of the wound already necrotic, or at times on areas of the outer, almost undamaged wound periphery where soiling from liquified exudate had occurred. Already evacuated screw-worm wounds in which no further *C. bezziana* restrike had taken place, were insome instances wholly infested by *L. cuprina* larvae.

What effect such secondary invasion might have had on the resident *C. bezziana* larval populations. or on the host animal itself was, not determined due to the treatment undertaken.

CONCLUSION

The establishment of *C. bezziana* as a permanent parasite in the Eastern Cape Province, after its continued occupation of these areas for the past seventeen years, must be accepted.

That little alteration in the limits of distribution of *C. bezziana* has occured following its initial rapid spread is in some respects a puzzling feature, considering the equally favourable environmental conditions existing further northwards along the Transkeian coastal region.

There can be little doubt as to the close interrelationship between screw-worm fly strike and multi-host tick activity in the coastal regions of the Eastern Cape. That these ticks play a major rôle in determining both the number of strikes occurring on cattle and the choice of site on the bovine body is obvious, and in the light of the results presented in this paper any form of control measure aimed at reducing screw-worm incidence should be directed as vigorously against tick life as against *C. bezziana* itself.

The suspected development of a strain of screw-worm flies tolerant to dieldrin is a factor not known to have occurred elsewhere within the African or Asian distribution of this parasite, and can be regarded as a disturbing circumstance.

SUMMARY

1. The introduction of the Screw-worm Fly, *(Chrysomya bezziana* Villeneuve), to the Eastern Cape Province and its spread throughout this area, is discussed.
2. Data is presented on the seasonal occurrence of the fly, and on the rôle played by various multi-host tick species in determining both the incidence of strike, and the site choice on bovines.
3. The development of a strain of *C. bezziana* tolerant to the chlorinated hydrocarbon compound, dieldrin, is suspected.

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Jl S. Afr. vet. med. Ass. 39 (1): 13—15 1968

A NOTE ON THE DISTRIBUTION OF CREATINE PHOSPHOKINASE (CPK) ACTIVITY IN SHEEP

J. M. M. Brown and Adriana M. Wagner*

SUMMARY

Creatine phosphokinase activity has been studied in various tissues of the sheep apparently for the first time. Very high levels were found in skeletal muscle and lower but none the less appreciable levels are present in structures containing smooth muscle, in nervous tissues and in the adrenal glands. Negligible activity was found in parenchymatous, glandular and lymphatic tissue. Blood levels in the sheep are mentioned.

Increased plasma CPK activity in sheep will most likely result from myopathy, but inflammatory or degenerative conditions of nervous tissue, the spleen or adrenals must be borne in mind in this regard.

INTRODUCTION

Creatine phosphate is a store of energy-rich phosphate which is used for the replenishment of ATP by means of the Lohmann reaction, catalyzed by creatine phosphokinase (CPK) [1, 2].

ADP + Creatine phosphate $\xrightarrow{\text{CPK}}$ ATP + Creatine. Creatine phosphate is thus part of an energy storing device which makes it possible for an organ to go rapidly into action and perform a large amount of work in a very short time. It is thus present in the striated, smooth and cardiac muscles of all classes of vertebrates but is absent from those of invertebrates [2]. The larger and more powerful skeletal muscles contain larger amounts of creatine phosphate than the slower acting smooth muscle of the gastro-intestinal tract (which may contain as little as one-fifth of that present in skeletal muscle) [3]. Skeletal muscle in which activity is irregular contains about four times as much creatine phosphate as ATP. In cardiac muscle, which is constantly in action, ATP and creatine phosphate are present in nearly equal amounts [1], but these are far less than those encountered in skeletal muscle [2].

Creatine phosphate is also present in nervous tissue, spermatoza and the electric organs of certain fishes, e.g. *Torpedo* sp. These electric organs, which can go into action almost instantaneously and dissipate large amounts of energy in a short time, are the only known structures containing concentrations of creatine phosphate comparable to those in striated muscle [2, 3, 4].

The diagnostic use of plasma creatine phosphokinase (CPK) activity levels has received much prominence in the literature pertaining to the laboratory diagnosis of myodystrophic diseases in man [5, 6, 7, 8]. Increased plasma levels of the enzyme are taken as evidence of increased muscle tissue breakdown. The use of plasma CPK levels in the diagnosis of ovine myopathy has recently been reported by Clark [9], Clark & Wagner [10] and Brown [11]. Cardinet, Littrell & Freedland [12] have investigated plasma levels of CPK in equine paralytic myoglobinuria, while Gerber [13] has also reported on its plasma levels in equine myopathic and cardiopathic conditions.

Normal plasma levels of CPK activity in Merino sheep have been statistically established as being 3-17 Sigma units (80% range). The 10% upper limit of normality is 32 Sigma units [14].

We are presenting in this paper data indicating the distribution of CPK activity in sheep tissues. These data thus indicate the reliability of determinations of plasma CPK levels in the diagnosis of ovine myopathy.

MATERIALS AND METHODS

A clinically normal adult Merino wether was slaughtered by exsanguination, skinned at once and used for sampling of the various tissues indicated in Table 1 below. All the

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samples were collected within half an hour of slaughter. All tissues were rapidly freed from adhering fat and serous membranes and then briefly washed in running cold water. Ten percent tissue homogenates in ice cold 0.25M sucrose were prepared, using a high-speed stainless homogenizer. † The homogenizing chamber of the instrument was immersed in ice during disruption of the tissues concerned. Where muscle tissue is described in Table 1 below as belonging to a particular muscle group, representative samples of the group concerned were removed from the limbs concerned and homogenized together.

Immediately after preparation all homogenates were placed in a refrigerator and kept there until used for the assay of CPK activity. All the assays reported below were completed within two hours of the slaughter of the animal.

CPK was determined according to the colorimetric procedure outlined in the Sigma technical bulletin No. 661, using the appropriate reagent kit supplied by the Sigma Chemical Co. (St. Louis, Mo.). Optical density readings were made in a Unicam S.P. 500 spectrophotometer at the wavelength specified in the method.

RESULTS

These are tabulated below.

DISCUSSION

The levels of CPK activity in sheep tissues fall into three distinct groups. Highest levels of activity were found in the skeletal muscles as expected, particularly in those muscle

Table 1: CREATINE PHOSPHOKINASE ACTIVITY IN VARIOUS SHEEP TISSUES

(Values are given in Sigma units per 100 mg of wet tissue).

Tissue	Units	Tissue	Units
M. rectus abdominis	1748	**M. pectoralis descendens**	1468
M. longissimus dorsi	1588	**M. deltoideus**	1428
M. quadriceps femoris	1548	**M. biceps brachii**	1328
M. triceps brachii	1508	**M. masseter**	1328
M. iliopsoas	1508	**M. temporalis**	1168
Mm. gluteus	1468	Body of tongue	1112
M. gastrocnemius	820	Cerebrum	756
Diaphragm	648	Spinal cord	508
Omasum	472	Cerebellum	344
Reticulum	420	Ventricular myocardium	232
Rumen	368	Spleen	236
Abomasum	232	Adrenal (whole)	240
Duodenum	272	Abdominal aorta	156
Rectum	272	Urinary bladder	156
Kidneys	76	Lung	68
Pancreas	68	Prescapular lymph node	24
Liver	12	Distal colon	48

groups with a postural function. Smooth muscle structures comprise the majority in the second group. Levels of CPK activity equal to those found in the very active smooth muscle of the forestomachs, or in skeletal muscles such as the diaphram and gastrocnemius, are to be found in the nervous tissue of the brain and spinal cord. The ventricular myocardium also falls into this second group and contains CPK levels no higher than those in the smooth muscle of the abomasum and alimentary canal. It is noteworthy that the adrenal gland (cortex and medulla were homogenized together) contains appreciable amounts of the enzyme.

Parenchymatous organs of high metabolic activity, glandular and lymphatic tissue from the third group in which CPK activity is negligible. Lowest levels of CPK activity were found in the liver.

The data presented above are in excellent accord with the known distribution of creatine phosphate in animal tissues, shown in Table 2. This table has been compiled from data given by Baldwin [2] and West & Todd [3].

Gerber [15] has presented data regarding the distribution of CPK in the tissues of five horses. Our data compare very well with his in so far as skeletal muscle, nervous tissue, the pancreas and the liver are concerned. He found horse ventricular myocardium to contain about 30% less CPK than skeletal muscle. Ovine myocardium according to our data contains about 84% less CPK than skeletal muscle. On the other hand we found that the ovine spleen contains 83% less CPK than skeletal muscle, whereas the corresponding figure given for the horse is about 98.4% less [15].

The distribution of CPK in sheep and in horse tissues is similar to that reported for other species e.g. pigeon [16], rat [16,17], human [17, 18]. It can be prepared in good yield from ox brain [19].

Most CPK appears to be located in the cytoplasm of tissues which contain it in appreciable amounts, although significant amounts have been detected in mitochondria and microsomes [16, 17]. Isoenzymic forms of CPK have been isolated from these tissues [16, 19].

Apart from elevated levels of CPK activity found in patients with skeletal myopathy, increases in plasma CPK have been found in cases of myocardial infarction [5, 6, 8, 20]. Elevated plasma CPK levels are also a feature of hypothyroidism [21, 22, 23].

Muscular exercise may cause an increase in plasma CPK activity [5, 8, 18].

Essentially normal values are found in a wide variety of diseases of the central nervous system, e.g. anterior poliomyelitis, cerebrovascular disorders, motor neurone disease,

Table 2: DISTRIBUTION OF CREATINE PHOSPHATE IN THE BODY TISSUES OF ANIMALS.

(According to Baldwin[2] and West & Todd[3]. Creatine phosphate levels are given as mg%P).

Tissue	Animal	mg%P
Skeletal muscle	Cat, rabbit, rat, frog	50—80
Electric organ	**Torpedo** sp.	37
Brain	Dog	12
Sciatic nerve	Rabbit, frog	6—7
Myocardium	Rabbit, rat	5
Stomach	Rabbit, rat	3—4
Uterus	Rabbit	1.4
Testis	Rabbit	1.4
Spleen, kidney, liver	Rabbit	—

Parkinsonism, polyneuritis and tumours of the brain and spinal cord [5, 7, 8], while muscle atrophy of neurogenic origin raises the plasma levels scarcely at all.

From the data presented in this paper it seems logical to conclude that increased plasma levels of CPK in the sheep most likely will be the result of skeletal, smooth or heart muscle pathology. Until shown otherwise, we should bear in mind that inflammatory or degenerative conditions involving nervous tissue, the spleen and the adrenals may also cause similar elevations in plasma CPK activity in the sheep. Inflammatory or degenerative conditions involving the liver, kidneys, lungs, pancreas and lymph nodes are unlikely to increase plasma CPK activity.

ACKNOWLEDGEMENTS

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SURGERY OF BOVINE IMPOTENTIA COEUNDI

IV Stenosis of the *Praeputium* Excluding the *Orificium Praeputiale*

C. F. B. HOFMEYR*

SUMMARY

Twenty-five cases of stenosis of the parietal layer of the preputial skin, excluding the *orificium praeputiale,* are included in this group. This condition is either overlooked or literature misdiagnosed if one accepts the paucity of relevant as indicative of its supposed incidence.

Three bulls recovered with local massage; obstruction was simply caused by chronic oedema. One case was inoperable and its treatment is recorded as a failure.

Two bulls recovered after resection of the hard fibrous ring at the base of the glans. Of the remaining 19 bulls, all recovered after the application of one or more of five surgical techniques. The first two closely related techniques constituted praeputioplasty by incision.

Fourteen bulls were successfully treated in this manner. The other three techniques constituted praeputioplasty by excision and involved the amputation of a mass of granulation tissue and a section of the parietal layer of the prepuce. In one case praeputioplasty by incision was subsequently required to effect complete recovery.

The results prove that the conditions described are eminently amenable to treatment, in contrast to the poor prognosis accorded them in the occasional reference in the literature.

INTRODUCTION, INCIDENCE AND DEFINITION

This paper is one of a succession of publications [1,2] based on a study of surgical pathology of the penis in 176 cases [3]. Twenty-five of these suffered from stenosis of the parietal layer of the prepuce, excluding the preputial orifice.

The breed distribution of the bulls was: Friesland, 8 cases; Jersey, 6; Afrikaner, 6; Sussex, Hereford, Red Poll, Santa Gertrudis and Brahman, one each.

The high overall incidence indicates that stenosis of the preputial cavity is one of the commonest surgical conditions affecting the penis and adnexa of the bull, yet, as such, it is hardly mentioned in the literature. By contrast, lesions in the retropreputial area are accorded relatively frequent mention, in spite of the similarity in pathogenesis (see below). Stenosis of the prepuce represented the sole disabling lesion in this group and is presumably equally common elsewhere. On the other hand, not a single case of adhesion of the glans penis to the parietal layer of the prepuce was encountered. It is conceded that adhesions may occur where there has been extensive destruction of both the parietal and penile layers of the prepuce but such occurrences would be exceptional. It is contended that the supposed prevalence of adhesions is erroneous and is based on the confusion between what constitutes adhesion and what stenosis. The latter really is the responsible lesion. General — and incorrect — usage of the term "phimosis" to indicate a diseased condition instead of a symptom common to a number of pathological states, as well as lack of critical analysis of the underlying pathology and pathological physiology, must be held responsible for this confusion.

In referring to the literature, allowance has been made for this usage and the term placed in quotation marks when employed in the customary sense. The first article [1] of this series provides details of the terminology employed.

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AETIOLOGY AND PATHOGENESIS

"Phimosis", as generally employed, is stated to be due to "congenital adhesions"[4] or acquired causes[4, 12]. The latter group is regarded as being more common[4]. It may follow on wounds, bruising and necrosis of the sheath[4, 7], purulent infections[5, 9, 10, 12] injury by plant material like thorns and burrs[8] with consequent stenosis[4, 6] of the preputial lining or adhesions of the glans to the parietal layer[4, 6, 9, 10, 12] or the formation of large masses of fibrous tissue[5, 12], all of which interfere with the full movement of the glans relative to the praeputium.

The infections are attributed to streptococci, *Corynebacterium*[5], *Pseudomonas deruginosa, Proteus vulgaris, Spherophorus necrophorus, Actinobacillus ligniéresi, Mycobacterium tuberculosis*[9] and staphylococci[10].

Of the cases presently under discussion, three suffered from chronic oedema, which caused obstruction to erection: there was no cicatricial stenosis. The pathological process in the other cases had many features in common with the group described in a previous paper[2]. The development of granulation tissue succeeded by fibrosis was constant in both. Concomitant oedema featured much less commonly in this group than in the previous one. Only in two cases was it seen at the time of examination. As the bulls came to hospital such a long time after the injury — usually months later, the shortest period being "at least three weeks" — it is conceivable that any initial oedema might have had time to recede. The suggestion that concomitant oedema is not such a prominent feature in this group as in the previous one, is strengthened by the wellknown fact that owners tend to seek professional help much sooner when extensive swelling is noticed. It may be assumed, therefore, that the initial injury did not cause undue alarm because of relatively slight visible changes and that professional advice had been sought only after impotence due to cicatricial stenosis had become apparent.

In only three cases was a purulent infection present. This differs materially from the previous group[2], where the incidence was about one-third. In only one case was the discharge of blood from the preputial orifice reported. In none of the cases were scars seen on the parietal layer of the prepuce but small wounds can heal without visible scarring. The long interval elapsing since injury might have allowed the infection to clear up, but then no such information had been presented by the owners except in one case. The bruising and oedema in this group were usually less extensive than in the previous one and, therefore, may have presented a less favourable medium for bacterial proliferation. This possibility explains the infrequency of infection.

There can be no doubt that trauma had been the cause of the pathology. Where the history was explicit on this point, service injury was mentioned. As the site of the lesions in this group is more distal to those in the previous group[2], the conclusion is justifiable that the injuries were sustained either while the penis was malpositioned or before proper *introitus vaginae* had been effected. Consequently any lateral movement of cow or bull, or collapse of the cow, would be less likely to cause extensive injury. As this might indicate aberrant service behaviour, and as inexperience probably played a significant role here, a comparison of the age distribution of the bulls is interesting: three years and under, 16 cases; 3-4 years, five cases; older, four cases.

A similar age distribution as in the previous group[2] is thus evident. The relatively higher number of bulls of three years and under in this group as compared to the previous group cannot be regarded as significant: the number of cases is too low to allow of a definite conclusion.

DIAGNOSIS

From what has been stated, it is clear that the external appearances of stenosis of the preputial cavity are not spectacular. During the acute stage there may be some oedema but after this has subsided the external enlargement is so slight as generally to escape notice. The conclusive detection of the stenosis after pudendal block has been described before[1, 3]. The stenosis may be a thin ring (Fig. 1 : 1) or involve up to ten cm of the parietal layer of preputial skin (Fig. 1 : 2) because of a granulation tissue mass. Occasionally more than one stenotic ring may be present but this is usually only seen after the most distal stenosis has been alleviated. In a very small proportion of cases the granulation tissue may harbour a purulent infection

(Fig. 1:3). Finally the stenosis may take the form of a pseudocartilaginous ring surrounding the base of the glans.

In view of the differences of therapeutic approach, one must distinguish clearly between chronic oedema without any cicatricial stenosis, and stenosis due to the presence of granulation tissue in one form or another. The various forms of cicatricial stenosis, in turn, demand different operative techniques. The site and extent of the stenosing mass and the nature of impedance to movement of the penis must be determined accurately.

TREATMENT

Expectant treatment [13], irrigation of the sheath with various drugs [13], dividing adhesions surgically [4, 12] or by stretching the penis under epidural analgesia or general anasthesia [8] have been advised. In cases of chronic oedema as the only lesion, I have found applications of hot water, acriflavine-glycerine instillation into the prepuce and massage effective.

In cictatricial stenosis of the prepuce, surgery has been regarded as unsuccessful. In my opinion, there is only one effective treatment and that is operation under the influence of pudendal block and a tranquillizer (if necessary).

I have used the following surgical techniques, succeeded by daily post-operative instillation of acriflavine-glycerine into the sheath. For the sake of clarity, one must distinquish between (a) the outer or integumental layer of the prepuce, (b) the parietal layer or lining — on which all the ensuing operations are performed — and the penile layer.

Technique 1 (Fig. 1:4, 5) is used for removing a firm fibrous collar causing stenosis close to the base of the glans at the *fornix (fundus) praeputii.*

The tip of the penis is withdrawn as far as possible so that the fibrous ring, encompassing the glans close to its base, is fully exposed. A circular incision is made around the circumference of the penis immediately behind (i.e. proximal to) the collar. All blood vessels are either electro-coagulated or divided between ligatures as the incision is deepened to approach the *tunica albuginea.* A second circumferential incision is made in the same manner in front of the collar. The fibrous collar is cut through at one point and completely dissected off between the two circumferential incisions. The parietal layer of the prepuce is then joined to the edge of the penile layer by mattress or simple interrupted sutures using chromic catgut.

Technique 2 (Fig. 1:6, 7; Fig. 2:1) is applied in cases of simple ring stenosis of the parietal layer of the prepuce, where the opening only allows protrusion of part of the glans, so that the stenosed area forms a tight band round the penis. The penis is withdrawn as far as possible. After choosing an area free from large blood vessels, a longitudinal incision is made, usually dorsally but also laterally or even ventrally. The length of the incision varies according to the needs of the case but is on the generous side; usually five cm or longer. After cutting through the parietal layer of preputial skin, all the constricting fibres of the stenosing ring are divided, down to the level of the *tunica albuginea* if necessary. Meanwhile the penis is drawn out further to cause the incision to gape wider and to indicate whether the stenosis has been relieved completely. The wound is then closed by means of simple interrupted or mattress sutures using chromic catgut so that the suture line lies at right angles to the long axis of the penis.

Technique 3 (Fig. 1:7,8) is employed to correct ring stenosis if the opening prevents the tip of the glans issuing. By tissue forceps the stenosis is protracted and secured for the operation. An incision of suitable length — usually about three cm — is made through the ring and the double layer of the parietal layer of preputial skin and extended backward, whereupon it gapes like a double-layered V. Using simple, interrupted chromic catgut sutures, the cut edge of the preputial skin on the outside of the everted parietal layer is sutured to that inside, i.e. the two layers of each arm of the V are sewn together, leaving the arms free and separate. The glans is located and drawn through this previously stenosed area to ensure that complete freedom of movement is achieved. If so, the suture line is found to lie at right angles to the long axis, as it does in technique 2.

Technique 4 (Fig. 2:2-5) is applied whenever the cicatrical mass is so long and deep that the granulation tissue would still interfere with free movement of the glans even after the stenosis is relieved by section. In such cases withdrawal of the glans through the constricted portion is impossible. At best the *galea glandis* can be protracted.

The indurated and stenosing mass is delivered through the preputial orifice by alternate use of succeeding pairs of tissue forceps, so that the parietal layer of preputial skin becomes everted. A straight circumferential incision is made in a transverse plane behind (proximal) but close to the indurated mass. This incision only penetrates the superficial of the two layers produced by the manipulation. The uppermost part of the incision is deepened gradually. All large blood vessels are divided between ligatures. Eventually the inner layer is incised at this point. The cut end of the inner layer at the posterior edge of the original incision is secured by forceps and joined to the outer layer of the prolapsed sheath with a mattress or interrupted suture of No. 2 chromic catgut. The incision is gradually extended around the whole circumference. As soon as a short section of the inner layer has been incised, it is sutured to the corresponding position of the outer one in the manner described. This is continued until amputation is complete and the edges of healthy mucosa have been secured all round the circumference.

This technique is obligatory where the space is minimal between the posterior (proximal) border of the cicatricial tissue and the natural preputial orifice.

Technique 5 (Fig. 2:6). The indications for this method are in general the same as for technique 4. However, it demands that there be more space between the cicatricial mass and the natural preputial orifice. The only significant difference is that the circumferential incision is made not at right angles to the long axis but at an angle of 45 degrees. In the previous instance the suture line is a complete circle while in this instance it is oval.

Technique 6 (Fig. 2:7, 8). Here the indications coincide in general with those of the two previous operations. The significant differences lies in the circumferential incision, which is made in zig-zag fashion, so that, after completion of the operation, the suture line is coarsely serrated. This operation requires somewhat more delicacy in technique as well as enough room between the cicatricial mass and the natural opening of the prepuce.

EVALUATION OF SURGICAL TECHNIQUES

Technique 1 was used on two Afrikaner bulls having a stenosing collar of hard and dense connective tissue at the fornix immediately proximal to the glans, causing considerable thickening of the penis and preventing *introitus*. Both bulls regained sexual function.

The simplest form of stenosis is the ring type. Where the penis could be forced through the stenosing ring, a very simple operation was done (technique 2). Where the stenosed opening was too small, the V operation (technique 3) was employed; the appearance after the operation in both cases was the same.

Technique 2 was the primary operation done on 14 cases. It assured recovery without further operation in nine cases but had to be repeated once on three cases and twice in one case. One bull had two ring stenoses alleviated, one after the other, by means of this technique.

Technique 2, for reasons to be elucidated later was not regarded as a suitable primary operation in the other cases, but was employed successfully in two instances where a mild recrudescent stenosis had occurred.

One case was first operated upon according to technique 3. After healing it was seen that a fibrous tissue ring, much less serious than the first stenosis, hampered erection; operation according to technique 2 then was effective. In no instance did this mean that technique 2 should or could have been used initially.

One bull had undergone amputation of a part of the preputial linng (technique 6) for removal of a mass of granulation tissue, which had caused stenosis of the preputial cavity over some distance. After healing, a connective tissue ring was present, restricting erection. Final recovery was brought about by then employing technique 2.

It must be concluded, therefore, that the indication for operation according to tech-

nique 2 is the presence of a ring stenosis affecting the parietal layer of the preputial skin, provided that the connective tissue is not massive and that it is possible to evert the parietal layer, even partly, by pulling the flaccid glans through the stenosing ring. If applied to such cases, the technique is highly effective. In the four cases where the operation had to be repeated, it is possible that complete initial success would have been obtained if the operation had been somewhat more radical. However, one must caution against too long an incision; it can lead to significant shortening of the parietal layer of the prepuce on the one side of the penis with possible deformity of the erected penis. In all cases where the operation had to be repeated, the secondary stenosis were of decidedly lesser degree. In these cases, also, the subsequent operations were always performed at a new site.

Technique 3 is, in reality, an adaptation of technique 2, and is indicated where the employment of the latter technique is difficult or impossible because of certain mechanical features. Firstly, if the preputial orifice is so small as to completely or partly prevent the tip of the glans being protracted, the ring remains as a round cord after the operation and cannot be flattened against the body of the glans; secondly, in order to leave such a small opening the cicatrix is dense, thus also effectively resisting the telescoping effect when drawing the penis out. After completion of the operation, the appearance of the suture line is indistinguishable from that in the foregoing technique. Technique 3 was employed on two cases. In one a firm ring stenosis formed after amputation of part of the preputial lining; technique 3 assured final recovery. In the other bull, the latter technique caused a very definite improvement, although a recurring stenosis was relieved after applying technique 2.

Techniques 4 to 6 all have the same basic indications, viz. a stenosis more than about 1.5 cm in length, accompanied by a mass of granulation tissue which, if not removed, will form a continual impediment to erection even were the stenosis rectified. These techniques are thus all concerned with amputation of a portion of the parietal layer of preputial skin.

Technique 4 involves a circumferential incision at right angles to the long axis. Where there is little space between the mass involving the prepuce and the natural preputial orifice, this technique has to be employed. In the one case on which this was done, a second stenosis developed; this was rectified by using technique 2.

Although only one case was operated upon by technique 4, certain objections can be made against the technique on general principles. Putting in mattress sutures parallel to the cut edges inevitably causes drawing together of the tissues included in each suture. The completed circular suture line is thus immediately shorter than the normal circumference of the preputial membrane. In addition, the preputial cavity is actually a potential space, as the walls collapse when the penis returns to the prepuce. Healing of the incised tissues therefore tends to take place in a relaxed position. Some secondary stenosis is almost inevitable; admittedly, it can be rectified easily. To eliminate the constricting effect of parallel mattress sutures, those at right angles to the raw edges can be used. As the tissues included in the sutures make the suture thicker, this will probably again predispose to secondary stenosis. Interrupted sutures will do away with these objections, but are accompanied by another undesirable feature. As not only the parietal layer but also the rest of the wall of the preputial lining have to be sutured together, the sutures must be inserted some distance from the cut edge. As the layer itself is thin and soft, the edges are not easily secured in correct opposition: in this manner they tend to override — a feature which, obviously, is undesirable.

A search was made for an adaptation that would ensure a longer suture line. Techniques 5 and 6 were developed, although they require more space than technique 4 and cannot be applied if there is insufficient space between the cicatricial stenosing mass and the natural preputial opening when the penis is extruded.

Technique 5 involves changing the circular circumferential incision to an oval one by making the incision at about 45 degrees to the longitudinal axis. This was done in only one bull. It succeeded, inasmuch as normal functions was restored, despite a poor prognosis. The oblique incision automatically causes a material discrepancy in the length of the preputial wall when the most distal point of the oval is compared with the

most proximal one. This may not be important in breeds with excess preputial lining, like the Brahman, but conceivably can lead to some deviation of the penis in breeds in which the lining normally is just long enough, like the Jersey. Technique 5 is worthy of a further trial, particularly in bulls with copius epithelial folds, because it is simpler to perform than technique 6.

Technique 6 succeeds in lengthening the suture line by performing the amputation in zig-zag fashion. Accurate suturing is not as simple as in the two preceding techniques but the possible objection against technique 5 falls away in all breeds operated upon by technique 6, as it does not involve unilateral shortening of the preputial lining. It was the method employed in three bulls. Only in one bull was it necessary to resort to a second operation because of a slight secondary stenosis, a 3 cm incision being required.

All three amputation techniques resulted in final recovery of all the bulls. At the present stage of experience the least objection can be lodged against technique 6 in cases where it is applicable. Final comparative evaluation must await the completion of a larger series.

There was one failure which requires brief comment. The glans protruded from the prepuce most of the time. The formation of cicatricial tissue around the preputial cavity was so extensive that sharp surgery obviously had no advantage to offer. This only left massage, which proved ineffective, as the to and fro movement of tissues between the integumental and parietal layer of the prepuce was completely inhibited by the adhesions.

Post-operative infection occurred in only one case. Although the operations were done as aseptically as possible, complete asepsis was difficult to attain. This proves the excellent powers of healing of the preputial lining.

It can therefore be concluded that stenosis of the preputial lining is eminently responsive to operative surgery, for which six techniques are described.

Fig. 1 : 1 Stenosis of the prepuce distal to the *fornix praeputii* showing a ring stenosis.

1 : 2 Stenosis of the prepuce distal to the *fornix praeputii* showing a wide band of stenoing cicatricial tissue and fibrous tissue mass more distally.

1 : 3 Stenosis of the preputial mucous membrane by fibrous tissue and abscess formation. Some evacuated pus is seen below the prepuce.

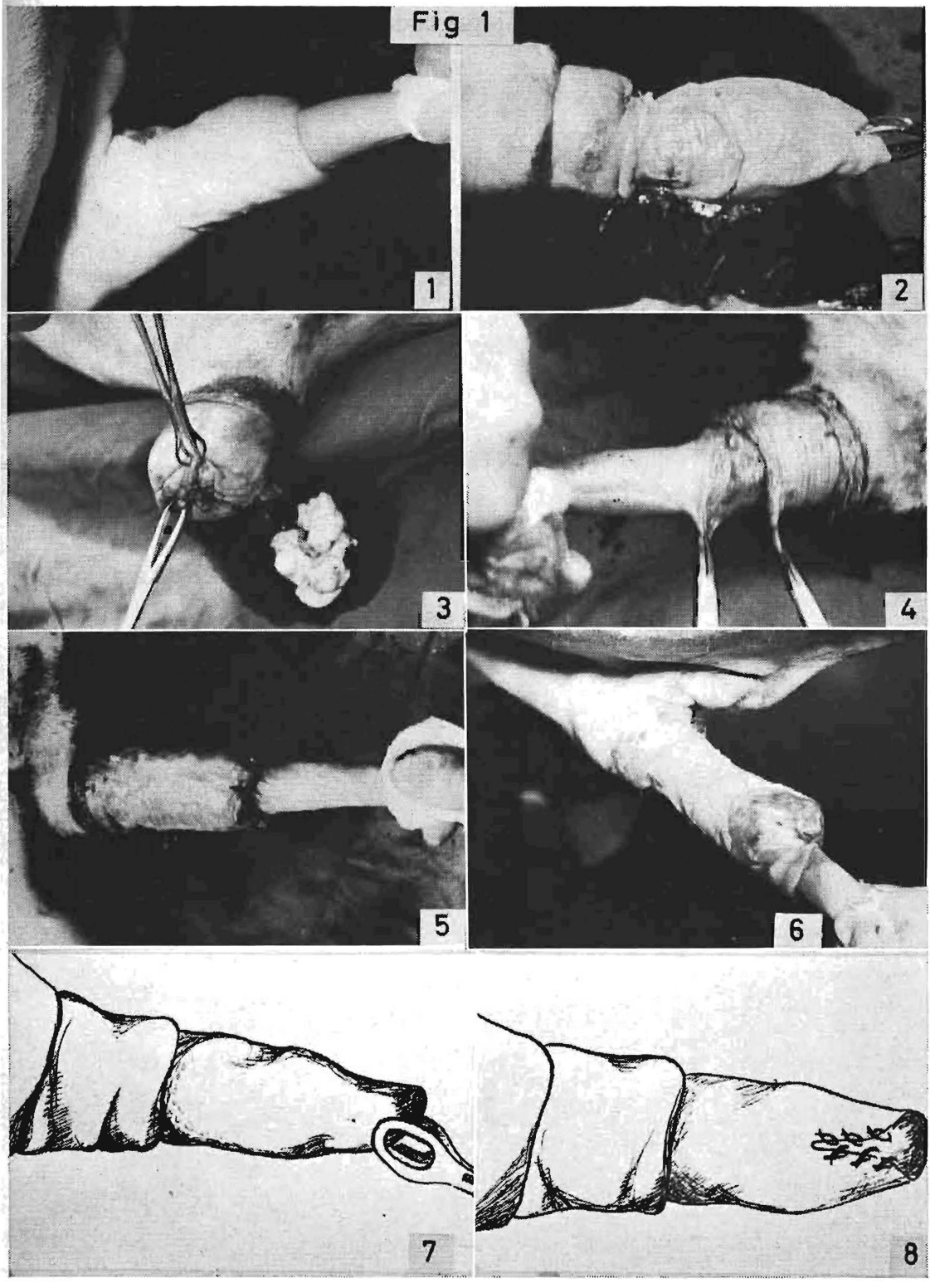
1 : 4 Stenosis of the prepuce at the *fornix praeputii* — the fibrous ring resected.
(a) Edge of the visceral mucous membrane.
(b) Edge of the parietal mucous membrane.

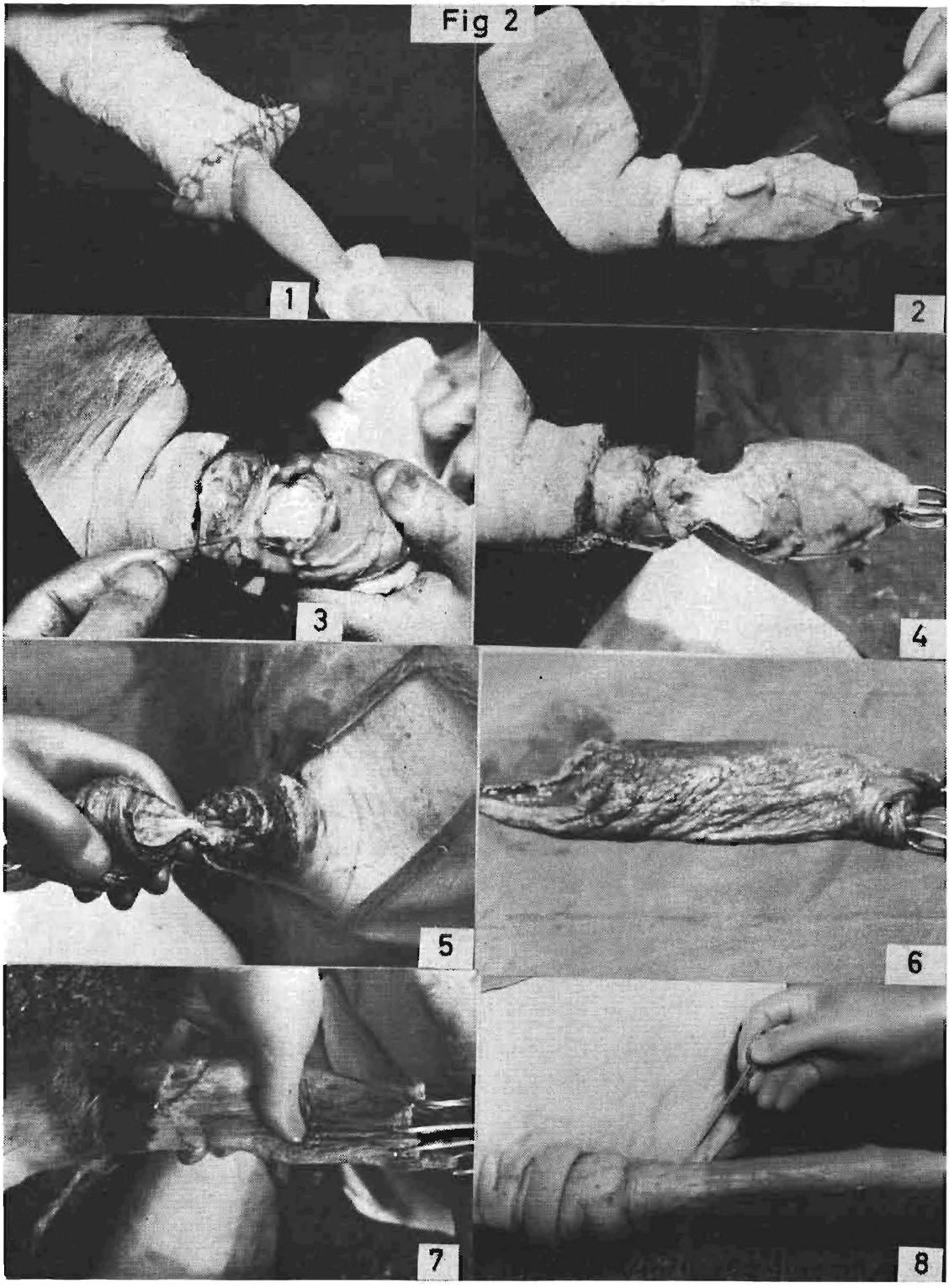
1 : 5 Stenosis of the prepuce at the *fornix praeputii.* Operation completed after suturing together the edges of the parietal and visceral mucous membrane.

1 : 6 Stenosis of the prepuce distal to the *fornix praeputii.* The longitudinal incision through all constricting fibres.

1 : 7 Stenosis of the prepuce-opening too small to let the *galea glandis* through.

1 : 8 Operation showing the gaping V incision and method of suturing. When the glans is drawn through afterwards, the suture line resembles that on **1** : 7.





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THE EPIZOOTIOLOGY OF NEMATODE PARASITES OF SHEEP IN THE HIGHVELD

I Worm Egg Counts in Lambs

R. J. THOMAS*

SUMMARY

The pattern of worm egg counts in lambs in a summer rainfall area is described. Infestation with three major species, *H. contortus, T. colubriformis* and *O. columbianum* appears to be strictly limited to the period of adequate rainfall, and pasture contamination dies out rapidly during the dry winter. The significance of these observations in the planning of parasite control measures is discussed.

INTRODUCTION

In 1958 studies on the seasonal incidence of nematode parasites of sheep were begun in selected areas of South Africa to obtain information on which to base more rational methods of controlling helminthiasis.

This paper reports the results of a two-year study on the seasonal fluctuations of nematode egg counts in lambs in the Ermelo district of the Eastern Transvaal highveld, an area with warm summers and cold dry winters. Lambs were used because they indicate the pattern of infestation in susceptible animals in the first season of exposure.

Data from other areas have been reported [1, 2, 3, 4].

MATERIALS AND METHODS

In May 1958, 100 grassveld Merino ewes with autumn born (April) lambs were purchased from a local flock and allocated in groups of 25 to four farms within a 40-mile radius of Ermelo. These are referred to as Groups 1, 2, 3 and 4, 1958-59. Groups 1, 2 and 3 were placed on pastures which had been grazed by sheep throughout the previous summer, while Group 4 was placed on pasture which had not been grazed for some years. A similar flock of 25 ewes with September-born lambs was also established on one farm to represent spring lambing (Group 5, 1958-59). As the ewes purchased in 1958 were in poor condition due to parasitism, they were replaced in July 1959 by fresh ewes with May-born lambs, referred to as Groups 1, 2, 3 and 4, 1959-60. Two groups of spring-born lambs, referred to as Groups 5 and 6, 1959-60, were also included. All the groups were treated as far as possible in the same way as commercial flocks in the area, being maintained on natural pasture with supplementary lucerne hay and cereals through the winter.

Throughout the period June 1958 to July 1960 differential worm egg counts were carried out on dung samples collected every two weeks from the flocks of lambs [6].

In some flocks treatment with phenothiazine or nicotine copper arsenate was necessary to prevent mortalities.

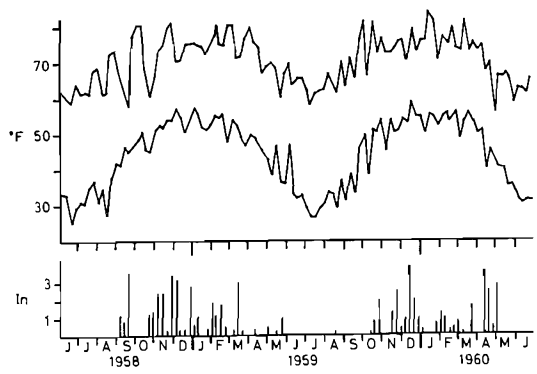

Fig. 1
Weekly variations at Ermelo in the mean maximum and mean minimum temperatures and total rainfall.

Maximum and minimum temperatures and rainfall were recorded throughout the survey period (Fig. 1).

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(Investigations were carried out while the author was on the staff of the Veterinary Research Institute, Onderstepoort.)

RESULTS

The following three nematode species were present in large numbers: *Haemonchus contortus* (Rudolphi, 1803), *Trichostrongylus colubriformis* (Giles, 1892), and *Oesophagostomum columbianum* (Curtice, 1890). *Strongyloides papillosus* (Wedl, 1856) and *Trichuris ovis* (Abildgaard, 1795), occurred in small numbers, while *Trichostrongylus falculatus* (Ransom, 1911) and *Bunostomum trigonocephalum* (Rudolphi, 1808) were rare. The dominance of the first three species was confirmed *post mortem*.

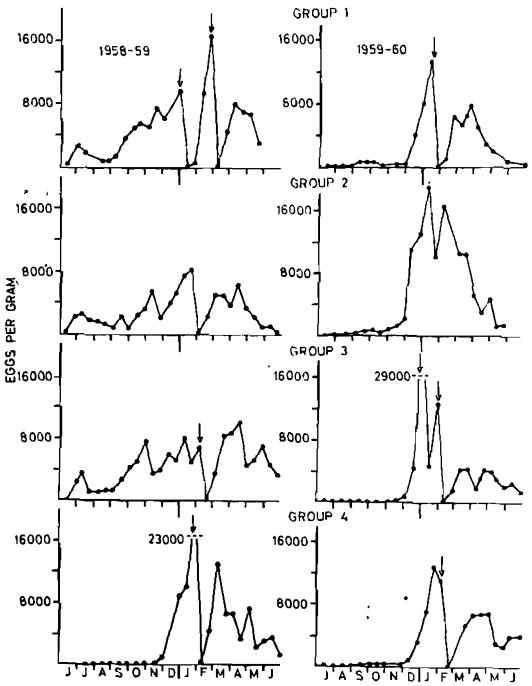

Fig. 2

Autumn born lambs. Variations in faecal worm egg counts of H.contortus
↓ = anthelmintic treatment.

Haemonchus contortus.

(a) Autumn-born lambs (Fig. 2).

The egg counts from the flocks placed on previously grazed pastures (Groups 1, 2 and 3, 1958-59) rose initially in June to 2,000 to 4,000 eggs per gram (e p g), fell in July and August and then rose again to a minor peak in October and then to maximum counts in December and January.

The dramatic effect of placing lambs on uninfested pasture was shown by Group 4, which was free of infestation throughout the winter and spring. Peak egg counts were noted at any time between December and the end of March, despite treatment. Thereafter the counts fell rapidly and by June-July had reached low levels.

In July 1959 these groups were replaced with young lambs. At this stage the egg counts were very low in all four groups and, although the lambs then went on to well-grazed pastures, they remained low until mid-November, when a very rapid rise occurred. The counts subsequently fluctuated at high levels for several months before falling again in May and June.

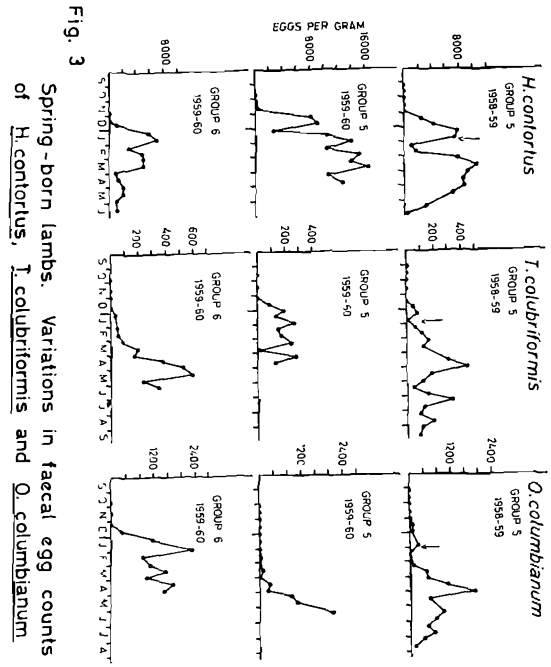

Fig. 3

Spring-born lambs. Variations in faecal egg counts of H.contortus, T.colubriformis and O.columbianum

(b) Spring-born lambs (Fg. 3).

A uniform pattern was noted in both years. Egg counts rose rapidly from the end of November, reaching 4,000 to 10,000 e p g. In Group 5 peak egg counts were recorded in March in both years.

Trichostrongylus colubriformis (Fig. 3 and 4).

As with *H. contortus*, Groups 1, 2 and 3 (1958-59) were infested in the winter and spring while Group 4 remained negative. Both autumn and spring-born lambs showed peak egg counts at any time from February to May. Anthelmintic treatment either with phenothiazine or nicotine copper arsenate had less effect on egg counts of this species than on those of *H. contortus*.

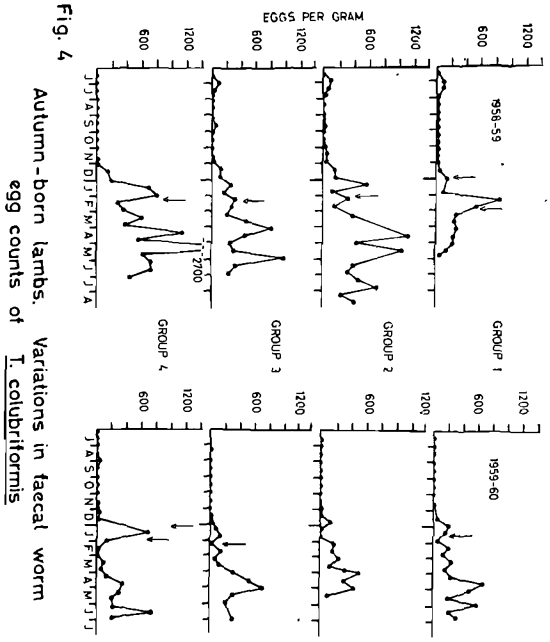

Fig. 4
Autumn-born lambs. Variations in faecal worm egg counts of T. colubriformis

Oesophagostomum columbianum (Fig. 3 and 5).

In neither autumn- or spring-born lambs were ova found until November. With the exception of Group 5 (1959-60), in which egg counts were still rising at the end of May, peak counts occurred at any time from January to April.

DISCUSSION

A general pattern of seasonal fluctuations in egg counts emerged which varied with the species, as follows:

Haemonchus contortus.

The cold, dry conditions in mid-winter on the highveld are apparently unsuited to the free-living stages of this species (Fig. 1). The lambs which were placed on the grazed pastures in May, 1958 were infested in winter by the infective larvae which had survived from the summer (Groups 1, 2 and 3, Fig. 2). The ewes of all groups were equally infested but few if any of the eggs deposited on the pasture developed to the infective stage in the winter. Otherwise the lambs in Group 4, which were placed on rested pastures, would have become infested before November. Furthermore, in 1959 the clean autumn lambs were only introduced to contaminated grazing in July and subsequently showed very low egg counts. This suggests that by this time the residual pasture contamination had also disappeared.

In both years a rise in egg counts developed in the lambs in late November. If a prepatent period of 18 to 21 days is allowed, this corresponds to a general infestation in late October to early November. This is closely correlated with the onset of regular rainfall from about mid-October and a rise in the weekly mean temperature to at least 60° F (15.5° C).

The egg count rose steeply in early December, leading to clinical signs of haemonchosis in December or January. The onset of this "haemonchosis season" was closely correlated with regular weekly rainfalls of over 1" (2.54 cm.) and mean weekly temperatures above 62° F (16.7° C) from mid-November. In view of the accumulating pasture contamination under the favourable weather conditions from December to March it would be expected that, despite treatment, egg counts would rise progressively over this period. This was not the case. A fall began in late March to April, suggesting that resistance develops which tends to limit any further rise in the egg count. This results in decreasing worm burdens in the surving animals, a situation

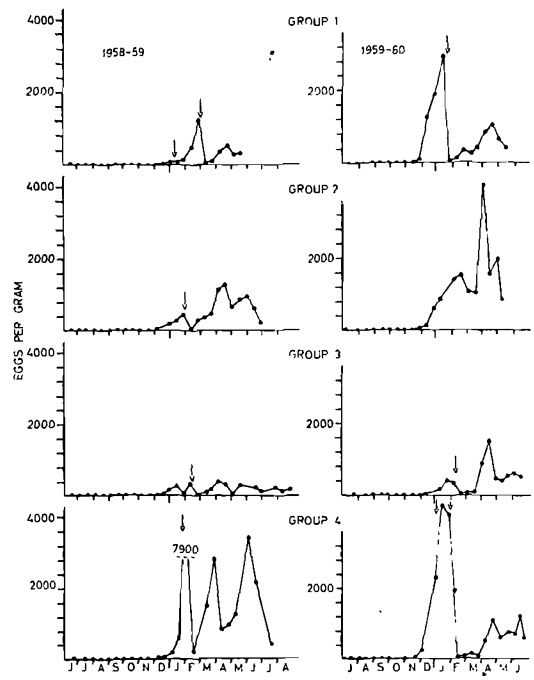

Fig. 5
Autumn-born lambs. Variations in faecal worm egg counts of O. columbianum

which is probably aided at a later stage by a decline in the rate of contamination in autumn.

A marked rise in egg counts occurred in spring (October) in lambs infested in the previous autumn and winter (Groups 1, 2 and 3, Fig. 2). This could not be attributed to fresh infestation because evidence from the spring-born lambs showed that egg counts only rose in late November (Fig. 3). The September rise in Groups 1 to 3 must, therefore, be due either to an increased egg output by an existing worm burden or to the maturation of larvae which have survived in the host since autumn. Thus a phenomenon closely related to the "spring rise" in ewes appears to occur in these lambs; this will be considered in more detail in a later communication.

These observations are similar to those reported from summer rainfall areas in the Cape Province [1, 2, 3]. These authors, however, were working with adult and yearling sheep and neither the onset of infestation in summer nor the effects of any previous exposure to infestation could be determined. Correlating their results, Reinecke [6] drew attention to the presence towards the end of summer of an increasing proportion of fourth stage larvae in the worm burden; this was attributed to host resistance. This observation supports the suggestion made earlier that the development of resistance in lambs is responsible for the levelling out of the egg count, and contributes to the fall in the autumn. These seasonal fluctuations in egg counts agree with observations in America [7] and Australia [8, 9]. It also confirms the general conclusion of Kates [10] that *H. contortus* has a low resistance to adverse climatic conditions and is particularly susceptible to drought and sub-freezing temperatures.

Dinnik & Dinnik [11] emphasized the importance of diurnal temperature fluctuations and stated that in the Kenyan highlands temperatures ranging from 54° - 74° F were optimum for the development of infective larvae. This is in general agreement with the temperature range at Ermelo during the haemonchosis season from November to April.

These marked temperature fluctuations do not occur in sheep-rearing areas in America, Australia and Britain and a mean monthly maximum temperature of 65°F (18.3° C) has been suggested as the lower limit for optimum development in these countries [8, 12, 13]. (Whether the lastt two authors are referring to mean maximum or mean monthly temperatures is not always clear). Silverman & Campbell [14] considered the "Dinaberg line" of 65° F to be inapplicable in Britain and showed that development proceeded at considerably lower temperatures. Variations in the requirements of the free-living stages however, may be the result of ecological selection [15].

Trichostrongylus colubriformis

Peak egg counts were noted in late summer and autumn, not in early winter as recorded by Barrow [1], Rossiter [2] and Viljoen [3]. The earlier peaks in the highveld may be due to the dry winters, as Roberts, O'Sullivan & Riek [16] state that monthly rainfall should exceed 76.2 mm and maximum temperatures vary from 12.8 to 18.3° C for optimum development.

Oesophagostomum columbianum

Peak egg counts were recorded at any time from January to May and interpretation is complicated by the fact that there is a long histiotrophic phase. Free-living stages are limited to the summer months, particularly during the high rainfall period. Rossiter [2] showed that fourth stage larvae were dominant from January to June, which supports this statement.

These observations can be applied to systems of worm control as follows:-

(1) Allow ewes to lamb down on pastures which have been rested the previous summer and autumn and, if necessary, treat the lambs with anthelmintics at the end of May.

(2) Dose lambs repeatedly with anthelmintics from the end of November to April because conditions for free-living stages are optimum during these months. A change to clean (winter rested) pasture following dosing should considerably reduce the rate of reinfestation and improve the effectiveness of the treatment.

(3) Dose ewes prior to lambing to decrease contamination of the pastures and subsequent infestation of their lambs.

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Jl S. Afr. vet. med. Ass. 39 (1): 33—44 1968

LABORATORY MASTITIS DIAGNOSIS:

The Microbiological Content of Parallel Teat and Gland Cistern Milk Samples from Quarters of Known Status

W. H. GIESECKE*, L. W. VAN DEN HEEVER†, D. C. HOPE* AND J. J. VAN STADEN*

SUMMARY

Assessment of the value of microbiological examination of "aseptically" drawn teat milk samples in the establishment of accurate mastitis diagnoses was the purpose of a study involving 120 udder quarters. After establishment of the status of the quarters by means of clinical and repeated (6x) intermittent cytological and biochemical examinations, a seventh teat sample was compared with a parallel gland cistern sample from each quarter. The data are provided in seven tables. No growth resulted from culture of 33.5 per cent of teat samples and 87.5 per cent of cistern samples. In the case of milk containing normal numbers of leukocytes, 60.8 per cent of cistern samples and only 26.7 per cent of parallel teat samples were culturally negative.

It is concluded that microbiological examination of teat milk samples as the sole means of establishing a positive mastitis diagnosis is rather unreliable even under the most ideal conditions of sampling. The necessity for simultaneous cytological evaluation and careful interpretation of results is emphasised.

INTRODUCTION

Besides traumatic, toxic or thermal causes, infectious agents which enter the udder via lactiferous or haematogenous routes are the most important in the aetiology of mastitis. Under suitably predisposing conditions nearly all organisms which localise in the udder are capable of inducing some degree of irritation which, when acute, may be marked by the cardinal symptoms of inflammation. Severe inflammation of the mammary gland of cows is comparatively rare. More commonly the condition exists without obvious symptoms but nevertheless accompanied by considerable changes in the glandular secretion, which becomes amber coloured or watery in appearance, altered in chemical composition and marked by inobvious or distinct deposits of cells and fibrin. These changes indicate that many, if not all, secretory cells of the gland have ceased their normal function, and that the secretion approaches the composition of blood plasma. Though the appearance of the secretion suggests acute inflammation, the lack of heat, pain and swelling causes the condition to be designated as subacute or even chronic, this state being reached by gradual transition from acute or peracute to chronic as partial regeneration takes place in the gland. The final stage may then be a chronic parenchymatous or interstitial inflammation with the secretory function of the gland being partially or completely normal. In the latter event, mastitogenic micro-organisms may still be present, and at any time their presence may lead to flare-ups or spread of infection to other quarters or animals. Such micro-organisms can also be found in milk samples taken with scrupulous asepsis from normal and apparently healthy lactating udders, as well as from udder tissue samples obtained from clinically normal lactating udders at slaughter. Similarly, both pathogenic and apathogenic microbes may inhabit the mammary glands of calves, pregnant heifers, dry and freshly calved cows without causing pathological changes[1-17]. Based on the results of such examinations, various authors have contended that:

(a) healthy udders may harbour a permanent "microflora" which includes inac-

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tive forms of the most important causes of infectious bacterial mastitis [3, 4, 18-26]; or

(b) the udder cistern, the teat cistern and teat canal are permanently inhabited by micro-organisms [27-30]; or

(c) the first milk fractions have a higher bacterial content than subsequent ones [24, 31-38]; or

(d) the last milk fraction contains only a small number of bacteria [39, 40], and may be sterile [41]; or

(e) only a small proportion of "aseptically" drawn samples are found to be completely free from bacteria [24, 25, 37, 39, 41-44]; or

(f) the originally sterile samples are contaminated when traversing the streak canal, or are contaminated by extraneous influences after being drawn from the udder [45-46].

Recent publications, however, indicate that most healthy udders are in fact sterile and only very rarely inhabited by micro-organisms [46-49]. On the other hand, the streak canal is almost always inhabited by large numbers of various microbes, and, as it cannot be sterilized, it is usually the primary source of contamination of milk samples.

Cytological and bacteriological examinations are considered the most effective and accurate methods for the detection of bacterial mastitis and asymptomatic infections of the gland tissue, and thus for the diagnosis and differentiation of secretory disturbances of the udder. Unfortunately, negligence and carelessness during the sampling decrease the possibility of obtaining the data essential for the rational treatment of single cases of mastitis or for the systematic control of the disease in herds by means of repeated laboratory examinations.

Generally, routine milk sample examination should consist of microbiological culture, bacterioscopic and cytological microscopy, additional indirect cytological methods, and of pH and chloride determinations. The final diagnosis is then made after due consideration of the results of the laboratory tests.

In the evaluation of microbiological data obtained in the examination of a milk sample, cognisance must be taken of the possibility that the specimen may have been contaminated. In order to determine the extent of such contamination, an investigation was made whereby samples of cisternal milk could be compared with corresponding teat milk samples obtained via the streak canal from quarters of which the status had been established by six pre-examinations.

MATERIAL AND METHODS

Thirty Friesians of the experimental dairy herd of the Veterinary Research Institute, Onderstepoort, were used in this study. The animals were kept under identical feeding and management conditions, and their ages varied from 30-132 months (mean 28.6). They had been in lactation for 1-32 months (mean 4) and their daily milk yield varied from 8-59 lb (mean 25.0). The cows were free from tuberculosis and brucellosis and appeared clinically healthy.

The udders and teats were subjected to detailed clinical examination. Before milking, the udder and teats were examined for asymmetry and ease of milk flow. After milking, a thorough manual examination of each teat cistern, tip and orifice, the gland cistern, the deeper regions of the secretory tissue, and the *Lnn. inquinales sup.* was carried out. With regard to the variations found in the consistency of individual quarters and teats, these were always compared with the corresponding ones on the other side.

No general or local antibacterial udder therapy had been applied to any of the cows during the three months preceding the investigation. The cows were milked twice a day in a two unit tandem milking parlour, using a machine of established efficiency.

The daily disinfection routine before commencement of the investigation was as follows: before application of the milk clusters, the lower halves of the udders were washed by hand with lukewarm water containing 200 ppm of available chlorine, the udders were dried with disposable paper towels and then milked; no post milking massage took place. After removal of the milk clusters, the teats were dipped into a 0.5% solution of Chlorhexidine gluconate B.P.

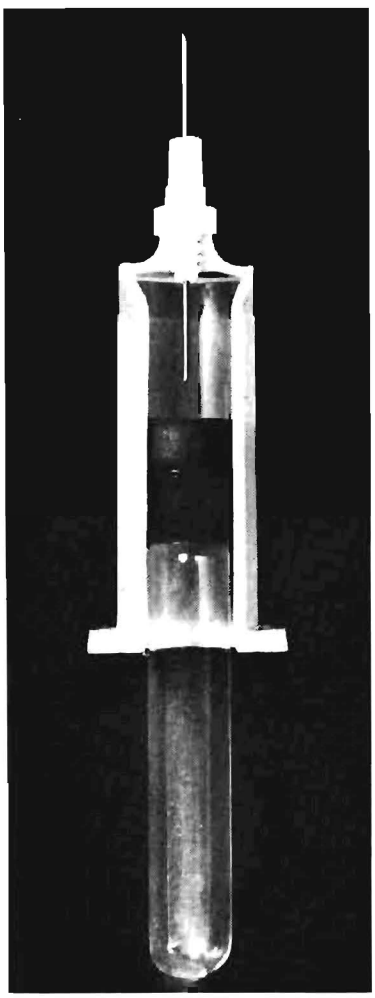
Sampling:

Immediately prior to experimental procedures the whole udder was further disinfected with a 15.0% solution of Chlorhexidine gluconate, the udder surface being left wet for 5 minutes. Only morning milk samples

were taken, and the cows were brought back into the normal milking routine in the late afternoon.

After disinfection of the udder, the teat tips and particularly the teat orifices were swabbed vigorously with a pledget of cotton wool saturated with 70 percent ethanol. Particular care was taken to avoid contamination of the teats by contact with hands, arms or clothing. After discarding the first three jets of milk, foremilk teat samples were taken directly into clean, dry, sterile, McCartney bottles held at an angle to limit the entry of extraneous matter. In the process of drawing the samples, care was taken not to touch the teat tip with the fingers or with the mouth of the bottle.

Fig. 1. "Venule" (barrel in section).



Sampling was carried out in the reverse order to swabbing. The bottles were filled to three quarters and the screw caps immediately replaced. Two separate samples, each comprising 50-60 ml milk, were obtained from each quarter and a single cistern sample of 5 ml was then drawn directly from the gland cistern [47-49], after puncture with a "Venule."* (Fig. 1).

To puncture the cistern the standing animal was held with a nose holder and the hocks tied. No anaesthesia or tranquillizer was used. A small horizontal snip was made by means of scissors into the skin at the site of puncture, at the base of the teat. Although horizontal surgical wounds of the teats are disliked and avoided, this small superficial wound was negligible. Retraction of the wound edges resulted in the wound opening sufficiently wide to render the use of forceps unnecessary and the needle of the "Venule" was inserted into the gland cistern through the wound. To avoid the rather long needle entering too deeply and causing damage by passing through the cistern into the opposite tissue in the event of the animal moving, the plastic cover of the needle was cut off so that only about 10 mm of the needle protruded beyond the collar so created. As the first drop of milk emerged from the butt, the vacuum container of the "Venule" was attached to the end of the needle, and 5 ml of milk drawn directly from the gland cistern [47-49]. After puncture the wound was swabbed with Chloramphenicol-gentian-violet tincture and 100,000 I.U. penicillin-streptomycin ointment was infused into each quarter. Observation at the next 2-3 milkings disclosed no complications.

Sample Examination:

The samples were immediately brought to the laboratory. One of the samples from each quarter was examined as follows: determination of pH, catalase and chloride content; neutrophile and total cellular content in triplicate Breed-smears of whole milk and microscopical evalution of cells, cell differentation and bacterial demonstration in triplicate sediment smears.

* Becton, Dickinson & Co. Columbus, Nebraska

The duplicate samples were stored at 4° C until used for whole milk and sediment inoculation onto blood tryptose agar and Wickerham stock culture medium [50]. Sediment for inoculation was obtained by centrifuging 10 ml of teat sample milk in stoppered centrifuge tubes and 5 ml of cisternal milk in the stoppered vacuum container at 3000 rpm for 10 minutes. Inoculated blood tryptose agar plates were examined after 18 and 42 hours incubation at 37° C. Where necessary, organisms were identified by the Bacteriological Diagnostic Laboratory of the Veterinary Research Institute, Onderstepoort. The inoculated Wickerham plates were incubated for 48 hours at 37° C and afterwards for 96 hours at 30° C. Cultures of yeast-like organisms were provisionally identified.

The pH of milk was determined electrometrically.

The catalase test was performed on 10 ml milk in graduated tubes with addition of 2.5 ml of a freshly prepared 3% v.v. solution of H_2O_2. The samples were incubated for 3 hours at 37° C [51].

The chloride content was determined by titration [46].

Three Breed smears from each quarter were subjected to parallel examinations of neutrophile and total cell content/ml milk. The average number of cells in three separate smears, made from each quarter sample and stained with Giemsa, was calculated from counts of all microscopic fields in horizontal and vertical central co-ordinates and the results rounded off to the last three decimals. Only clearly recognisable epithelial cells, lymphocytes, and neutrophiles were taken into account.

Three sediment slides were prepared from each quarter sample and 100 microscopical fields per smear were examined for cellular degeneration and clumping, and for bacteria and phagocytosis of bacteria [46, 53].

Efficiency of Laboratory Procedures:

The pH-meter readings showed a standard deviation of 2.5 percent over measurements of 100 aliquots of a milk sample.

The catalase test revealed a standard deviation of 4.9 percent in 50 aliquots of a sample containing a mean of 150,000 cells/ml milk and a standard deviation of 26.8 percent with 50 aliquots of the sample with a mean of more than 300,000 cells/ml milk. The chloride titration in 100 sample aliquots showed a standard deviation of 3.7 percent.

The control of Breed smear enumerations of the total cell content/ml milk was executed by examining 5 aliquots of a sample with a low, and 5 aliquots of another sample with a high cell content. A mean of 625 microscopical fields per aliquot with up to 150,000 cells/ml milk showed a standard deviation of 9.9 percent; the standard deviation was 23.7 percent for 5 aliquots of a sample containing between 300,000 — 500,000 cells/ml milk. Using the same samples as above, evaluation of the neutrophiles/ml milk revealed a standard deviation of 6.8 and 29.3 percent respectively.

Clinical terms used:

In the clinical description of the udder parenchyma the following definitions were used:

(a) *no fibrosis:* normal pliability and elasticity of the glandular tissue, tissue fine ly granulated and soft after milking (no pathological changes);

(b) *slight fibrosis:* a few single circumscribed coarse granulous indurations, or a single distinct induration of approximately 1-2 inches diameter;

(c) *distinct fibrosis:* tissue generally coarsely granulated and solid, multiple knotlike indurations, or a single distinct induration of approximately 2-3 inches diameter;

(d) *marked fibrosis:* tissue generally coarsely knotted, multiple extensive indurations replacing most of the elastic glandular tissue, diffusely solidified, or a large single induration.

In the clinical description of the cisterns the following definitions were used:

(a) *no fibrosis:* normal character of walls in all respects (no pathalogical symptoms);

(b) *slight fibrosis:* slight diffuse fibrosis, few slight indurations or a small distinct induration not interfering with function of cistern;

(c) *distinct fibrosis:* multiple slight indurations not interfering with function of cistern,

(d) *marked fibrosis:* multiple extensive indurations, or a large single induration disturbing the normal function of the cistern.

Table 1—CLINICAL STATUS OF 120 UDDER QUARTERS

Clinical findings	Parenchyma	Gland cistern	Teat cistern	Macroscopically normal milk	Milk watery, bluish, without floccules	Milk watery, bluish with small floccules	Milk showing some large floccules
No fibrosis	60.0%	72.5%	84.2%	55.8%	1.7%	—	—
Slight fibrosis	21.7%	15.0%	15.0%	18.3%	2.5%	—	—
Distinct fibrosis	6.7%	9.2%	0.8%	1.7%	6.7%	—	—
Marked fibrosis	11.6%	3.3%	—	—	7.5%	5.0%	0.8%

Table 2—ANALYSES OF CLINICAL AND CYTOLOGICAL FINDINGS IN SEVEN SUCCESSIVE TEAT MILK SAMPLES FROM 120 QUARTERS

Clinical classification of parenchyma	No fibrosis: 60.0%		Slight fibrosis: 21.7%		Distinct fibrosis: 6.7%		Marked fibrosis: 11.6%	
Corresponding cell counts/ml milk	$< 250 \times 10^3$	$> 250 \times 10^3$	$< 250 \times 10^3$	$> 250 \times 10^3$	$< 300 \times 10^3$	$> 300 \times 10^3$	$< 300 \times 10^3$	$> 300 \times 10^3$
	48.8%	11.2%	16.6%	5.1%	2.5%	4.2%	2.5%	9.1%

< = Less than
> = More than

Table 3—CLASSIFICATIONS OF CELL CONTENT OF SEVEN SUCCESSIVE TEAT SAMPLES FROM 120 QUARTERS

	$< 250 \times 10^3$ cells/ml milk	$> 300 \times 10^3$ cells/ml milk 29.6 ± 7.5%	
Mean and absolute deviation of fluctuations in cell content	70.4 ± 3.4%	$300\text{-}500 \times 10^3$ cells/ml milk 8.9 ± 4.6%	$> 500 \times 10^3$ cells/ml milk 20.7 ± 2.9%
Relative deviation from mean of fluctuation of cell content	4.8%	51.2%	14.0%
Mean and absolute deviation of repeatability of cytological evaluation	60.4 ± 5.5%	2.3 ± 1.6%	20.7 ± 2.5%
Relative deviation from mean of repeatability of cytological evaluation	9.1%	69.6%	12.0%

RESULTS

The data obtained in the course of six separate pre-examinations (samples A) of the herd on day 14, 12, 10, 7, 5 and 3 prior to puncture, from the teat samples (samples B) obtained immediately before cisternal puntures, and from the parallel cisternal puncture samples (samples C), are summarised in tables 1-7.

Clinical:

The results of the clinical examination of udder parenchyma, gland and teat cisterns, and the macroscopic character of the secretion are recorded in table 1.

Throughout the study the udders were repeatedly examined clinically but no changes could be detected.

Clinical and cytological:

The comparison between clinical status and the cytological content established during examination of samples A and B is furnished in table 2.

In the course of the seven consequitive examinations of teat milk samples from 120 quarters it became evident that fluctuations in the total cell content/ml milk were of a low order and the consistency of cytological findings in individual quarters was good in quarters which, according to the cell content/ml milk, were either healthy or severely diseased; fluctuation was highest and consistency poorest in quarters with critical cell counts lying between 300-500 x 10^3/ml milk

Cytological and bacteriological:

With regard to total cell content/ml milk and the absence of microbial growth in teat milk samples taken on seven consequtive occasions (A and B) and in cistern milk samples (C), the results are compared and summarised in table 4.

Table 4—SUMMARY OF TEAT AND CISTERN MILK SAMPLES FROM 120 QUARTERS SHOWING NO GROWTH ON MICROBIOLOGICAL CULTURE

	Cell content/ml milk	$<250 \times 10^3$	$> 300 \times 10^3$
Absence of bacterial growth	Teat Samples A	24.2%	11.5%
	Total	35.7%	
	Teat Samples B	22.5%	11.0%
	Total	33.5%	
	Cistern Samples	60.8%	26.7%
	Total	87.5%	

A summary of the various micro-organisms isolated in the course of examining samples A, B and C is given in table 5.

Data concerning the frequency and sources of micro-organisms isolated as well as the clinical and cytological status of the individual quarters concerned are given in table 6.

Table 5—CELL CONTENT AND DETAILS OF TYPE AND FREQUENCY OF MICROBIAL ISOLATIONS FROM TEAT AND CISTERN MILK SAMPLES FROM 120 QUARTERS

Code No.	Micro-organisms isolated	Frequency of positive microbial isolations from milk samples with $< 250 \times 10^3$ cells/ml:					Frequency of positive microbial isolations from milk samples with $> 300 \times 10^3$ cells/ml:				
		Teat Samples		Cistern Sample	Consistency of isolation from samequarter during		Teat samples		Cistern Sample	Consistency of isolation from same quarter during	
		A	B	C	A and B	A and C	A	B	C	A and B	A and C
1.	**S. epidermidis**	34.9	11.0	—	3	—	13.0	6.0	1.0	4.0	1.0
2.	**S. aureus**	2.4	3.0	—	—	—	9.3	9.0	9.0	9.0	9.0
3.	**Micrococcus** spp.	0.8	4.0	2.0	1	—	0.4	—	—	—	—
4.	**Sc. dysgalactiae**	6.7	—	—	—	—	7.1	3.0	3.0	3.0	3.0
5.	**Sc. faecalis**	3.8	—	—	—	—	2.8	—	—	—	—
6.	**Sc. bovis**	2.6	—	—	—	—	—	—	—	—	—
7.	**Sc. zooepidemicus**	0.5	—	—	—	—	0.2	—	—	—	—
8.	**Sc. cremoris**	0.2	—	—	—	—	—	—	—	—	—
9.	**Bacillus** spp.	4.9	1	—	1	—	0.7	—	—	—	—
10.	**E. coli**	8.0	2	—	—	—	2.9	—	—	—	—
11.	**Pseudomonas** spp.	—	37.0	—	—	—	0.2	25.0	—	—	—
12.	**Alcaligenes faecalis**	0.6	—	—	—	—	0.2	—	—	—	—
13.	Yeast-like organisms	1.8	—	—	—	—	0.4	1.0	—	—	—
14.	**Proteus vulgaris**	0.6	—	—	—	—	—	—	—	—	—

A: Mean of 6 pre-examinations of teat samples.

B: Single teat milk sample taken at time of cisternal puncture.

C: Single cistern milk sample obtained via cisternal puncture.

Table 6—FREQUENCY AND TYPE OF MICROBIAL ISOLATIONS FROM TEAT AND CISTERN MILK SAMPLES RELATIVE TO THE CLINICAL STATUS OF 120 QUARTERS AND THE CYTOLOGICAL STATUS OF THEIR SECRETION.

Clinical State of Quarter and Cytological State of Secretion	Details and Frequency of Microbial Isolations: A: Teat milk samples (mean of 6 pre-examinations)														B: Teat milk samples							C: Cistern milk samples			
	S. epidermidis	S. aureus	Micrococcus spp.	Sc. dysgalactiae	Sc. faecalis	Sc. bovis	Sc. zooepidemicus	Sc. cremoris	Bacillus spp.	E. coli	Pseudomonas spp.	Alcaligenes faecalis	Yeast-like org.	Proteus vulgaris	S. epidermidis	S. aureus	Micrococcus spp.	Sc. dysgalactiae	Bacillus spp.	E. coli	Pseudomonas spp.	S. epidermidis	S. aureus	Micrococcus spp.	Sc. dysgalactiae
No fibrosis																									
Milk < 250×10³ cells/ml.	25.3	1.5	0.6	4.0	2.4	2.2	0.3	—	3.3	5.6	—	0.4	1.2	0.6	7.0	1.0	3.0	—	—	1.0	22.0	—	—	2.0	—
Milk 300-500×10³ cells/ml.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—	5.0	—	—	—	—
Milk > 500×10³ cells/ml.	6.5	0.3	—	1.0	1.5	—	—	—	0.2	0.8	0.2	—	—	—	3.0	—	—	—	—	—	9.0	1.0	—	—	—
Slight fibrosis																									
Milk < 250×10³ cells/ml.	7.7	0.8	0.2	2.2	1.0	0.4	—	—	0.9	2.2	—	0.2	0.6	—	3.0	2.0	—	—	—	1.0	9.0	—	—	—	—
Milk 300-500×10³ cells/ml.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—	—	—	—	—
Milk > 500×10³ cells/ml.	2.2	0.5	0.2	0.2	0.4	—	—	—	—	0.5	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—
Distinct fibrosis																									
Milk < 250×10³ cells/ml.	0.8	—	—	—	0.2	—	—	0.2	0.3	0.2	—	—	—	—	—	—	—	—	—	—	3.0	—	—	—	—
Milk 300-500×10³ cells/ml.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.0	—	—	—	—	2.0	—	—	—	—
Milk > 500×10³ cells/ml.	0.5	4.0	0.2	0.3	0.2	—	—	—	—	0.3	—	0.2	0.2	—	1.0	1.0	—	—	—	—	1.0	—	4.0	—	—
Marked fibrosis																									
Milk > 250×10³ cells/ml.	1.1	0.2	—	0.5	0.2	—	0.2	—	0.4	—	—	—	—	—	1.0	—	1.0	—	1.0	—	3.0	—	—	—	—
Milk 300-500×10³ cells/ml.	1.4	—	—	0.2	0.7	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Milk > 500×10³ cells/ml.	2.1	4.5	—	3.3	—	—	0.2	—	0.5	1.4	—	—	0.2	—	1.0	5.0	—	3.0	—	—	7.0	—	5.0	—	3.0

DISCUSSION

As shown in tables 5 and 6, the frequency of microbial isolation from various types of samples and the nature of micro-organisms isolated, varied considerably. Only a few of the same organisms were consistently present, (more than four times during 8 examinations), thereby indicating their established presence in the quarters concerned. In connection with the micrococci isolated from the cisternal samples, neither clinical nor laboratory examinations (table 7) disclosed any evidence of their pathogenicity. There was also no positive correlation between these isolations and previous examinations of teat samples, so that these micrococci appear to have been carried into the milk samples by contaminated puncture needles.

On the other hand, Henderson [54] states that "the variety of micro-organisms found in the normal and diseased udders indicates that the bacteria found there depend wholly on the accident of their advent from the external environment, and upon their power to maintain their existence either as epiphytes or as pathogenic forms", and the possibility of micrococci being saprophytes in the gland cistern cannot be completely excluded.

Concerning the single quarter from which *Staphylococcus epidermis* was isolated, the cell content of over 500 x 10^3 cells/ml milk indicated distinct abnormality. No cocci or phagocytosis of micro-organisms were detectable, however, in the sediment, while the catalase readings (11.0 vol. %), the pH (6.70), and chloride content (0.0745g/100 ml milk) were within physiological ranges and far below the group means of catalase 40.50 ± 11.0 vol. %, pH 6.83 ± 0.10, and chloride 0.906 ± 0.0029 g/100 ml milk.

The comments on *Micrococcus* spp. also apply to the repeated isolation of *S. epidermidis.*

In the 12 quarters with distinctly abnormal cell counts (over 500,000 cells/ml milk) as well as distinctly increased catalase readings, chloride content and pH (table 7), only *Staphylococcus aureus* and *Streptococcus dysgalactiae* could be found (tables 5 and 6). The absence of acute clinical symptoms indicate *galactophoritis* and *mastitis catarrhalis chronica* caused by *S. aureus* in 9 quarters, and by *Sc. dysgalactiae* in 3 quarters. The micro-organisms isolated from the other quarters during the different stages of the study must be regarded as contaminants. Nevertheless, as they may be pathogenic for the mammary gland, they might easily have resulted in a confusing variety of positive "mastitis diagnoses" had one relied on only a single examination. By contrast, consideration of the results of catalase, pH and chloride determinations in conjunction with the bacteriological, cytological and clinical examinations, and by using accepted norms on which a reliable diagnosis may be based, conclusions could be drawn relative to the aseptic or non-specific secretory disturbances of some of the quarters. The cows yielding only a little milk and being at the end of lactation had already been excluded.

The number of cows used in the study is too small to permit any significant conclusions. The results may, however, provide an indication of the relative efficiency of single and combined methods used in mastitis control work. Provided the stage of lactation of the cows is taken into account, it would appear from the degrees of fluctuation and repeatability of results that a direct cytological examination has considerable merit after an initial clinical examination.

The importance of the cell content, and particularly the leukocyte content, is well-known in mastitis work [55, 56]. In spite of its known inaccuracies [52, 57-59], the conventional Breed method has been used for assessing the accuracy of other methods in mastitis diagnosis [60, 61]. Direct counting unfortunately involves tedious microscopy and this has led to its elimination from routine procedures or to the counting of fewer microscopic fields, a step which invariably further increases the degree of inaccuracy. Recent development of electronic methods [59, 62-66], however, promises to meet the requirements of greater accuracy and speed, and to make possible intensive mastitis work on a large scale.

In the meantime, more attention should be given particularly to proper sampling by the usual method. It is well known that samples sent for microbiological mastitis diagnosis are rarely suitable. Sampled indiscriminately in unhygienic fashion, carelessly labelled, and milked into a variety of con-

Table 7—RESULTS OF CYTOLOGICAL AND BIOCHEMICAL EXAMINATIONS OF TEAT SAMPLES FROM 120 QUARTERS

Clinical status of quarter	Less than 250×10^3 total cells/ml							More than 300×10^3 total cells/ml						
	Biochemical Examination			Cellular content/ml of milk. Breed milk smear (*All absolute figures* × 1000)				Biochemical Examination			Cellular content/ml of milk. Breed milk smear (All absolute figures × 1000)			
	Catalase Vol %	Chloride g/100 ml milk	pH	Epithelial cells	Lympho-cytes	Neutro-philes	Total cell content	Catalase Vol %	Chloride g/100 ml milk	pH	Epithelial cells	Lympho-cytes	Neutro-philes	Total cell content
No fibrosis	8.90 ±3.73	.0788 ±.0026	6.56 ±.06	20.4	15.6	47.4	83.4	40.50 ±11.0	.0906 ±.0029	6.83 ±.10	50.0	72.5	190.8	313.5
				24.5%	18.7%	56.8%					15.6%	23.2%	60.8%	
Slight fibrosis	12.70 ±4.15	.0771 ±.0169	6.50 ±.10	20.3	21.9	71.3	113.5	36.90 ±22.00	.0896 ±.0011	6.83 ±.10	25.8	33.9	322.1	381.9
				17.3%	19.4%	62.9%					6.7%	9.0%	84.4%	
Distinct fibrosis	6.00 ±5.3	.0840 ±.0160	6.59 ±.12	28.6	19.8	59.3	107.8	78.20 ±13.75	.1182 ±.0054	7.23 ±.13	NCD*	NCD	NCD	NCD
				26.5%	18.4%	55.1%					NCD	NCD	NCD	
Marked fibrosis	23.65 ±14.35	.0987 ±.0183	6.60 ±.22	20.6	20.7	99.0	140.4	79.90 ±15.5	.1122 ±.0163	7.13 ±.20	NCD	NCD	NCD	NCD
				14.6%	14.8%	70.5%					NCD	NCD	NCD	

*NCD = no count and differentiation possible (cells too densely clumped).

tainers, the samples are either already sour, show macroscopical evidence of sediment and dirt, furnish evidence of heavy contamination when incubated on media, or contain antibiotic residues when they reach the laboratory. When taking milk samples for bacteriological examination it should be borne in mind that the result of a laboratory test is no better than the sample, and the sample is no better than the way in which it was drawn. It would be a grave error to think that bacteriological examination methods are invariably capable of isolating significant micro-organisms from material containing numerous contaminants and/or inhibitory substances. With few notable exceptions even the use of selectively inhibitory substances in sample bottles and culture media will fail when contaminating bacteria exceed a certain number. It should therefore be understood that if a laboratory is to carry out careful and conscientious mastitis diagnoses with correct interpretation and effective advice and proposals for the control of mastitis, the milk samples have to be taken and treated as carefully as possible. If they are not examined immediately, they must be kept well chilled until examined.

CONCLUSION

From the results of this investigation it is apparent that even under the most favourable conditions of sampling via the teat canal, the opportunities for microbiological contamination are so numerous that they seriously jeopardize the value of a single bacteriological examination. For obvious reasons, cisternal milk samples, obtained by a method such as used in this study, cannot be applied in practice. On the other hand, the value of direct cytological examination of samples is undeniable, and presents intensive organised veterinary control of udder health with a reliable and readily performed routine method. Consideration of the symmetry of the cell contents of the milk from the four quarters, and of the stages of lactation of the cows, is important in distinguishing the pathological from the physiological. The results of bacteriological examination, however essential for determining the nature and characteristics of microbial mastitogens, must always be interpreted with the greatest care and can only be judged with full cognisance of the cytological and clinical state of the quarter.

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PLASMA LEVELS OF CREATINE PHOSPHOKINASE ACTIVITY IN THE MERINO SHEEP

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SUMMARY

Normal values have been established for the activity of creatine phosphokinase in the blood plasma of Merino sheep as maintained at Onderstepoort. These values are somewhat wider than those given for human patients, especially in the 10 percent upper limit of the normal range.

INTRODUCTION

The use of plasma creatine phosphokinase (CPK) activity levels is an established tool in the clinical laboratory diagnosis of myopathy and in particular myodystrophic diseases[1, 2]. Its use in the diagnosis of muscle lesions in animal patients has recently been reported by Clark[3], Clark and Wagner[4] and Cardinet, Littrell & Freedland[5]. Determinations of plasma CPK activity may be of value in the presumptive diagnosis of bluetongue in the post-febrile and post-viraemic periods[4]. The possibility of correlating plasma enzyme levels with the virulence of strains of bluetongue virus intended for use in vaccine production has been mentioned[4]. The purpose of the investigation reported here was to establish the normal plasma levels of activity of the enzyme which could be expected in the Merino sheep used for the studies on bluetongue just cited.

MATERIALS AND METHODS

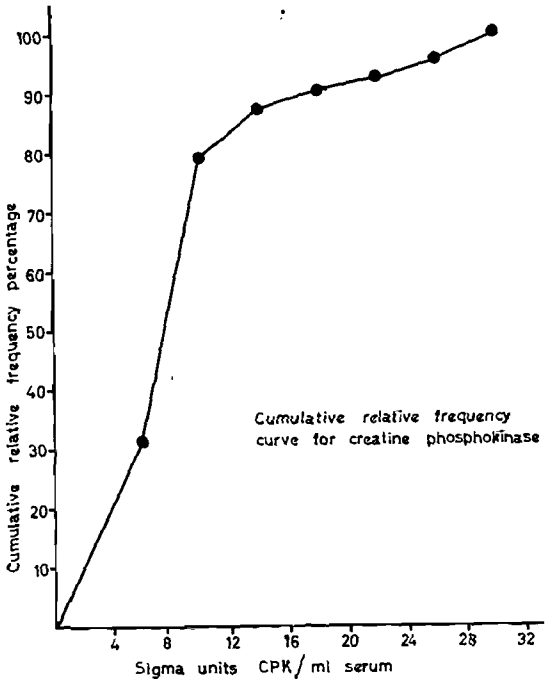
The animals used for this study were fully grown Merino sheep drawn from the pool of animals available at Onderstepoort. Their origin, nutrition and management were as reported previously[6]. The method of statistical evaluation of the data collected was also as described previously[6, 7].

Levels of plasma CPK activity were assayed according to the method outlined in the Sigma technical bulletin No. 661[8] and use was made of the diagnostic reagent kit No. 661 supplied by the Sigma Chemical Co.** for performing the assays. The trichloracetic acid solutions required were prepared from analytical reagent grades of this chemical. Photometric readings were made with a Unicam SP 500 spectrophotometer at 660 m u. Heparin was used throughout as the anticoagulant in the collection of blood samples.

RESULTS AND DISCUSSION

The cumulative relative frequency curve and histogram constructed from the data obtained, are presented as figures 1 and 2 respectively and the conclusions drawn from these curves are indicated in Table 1. Units of activity are as defined in the original procedure. The 80% range of 3-17 Sigma units

Fig. 1



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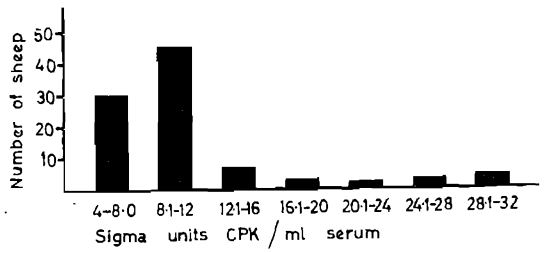

Fig. 2

Histogram of creatine phosphokinase levels of normal sheep.

Table 1—RANGES FOUND FOR CREATINE PHOSPHOKINASE (CPK) IN OVINE BLOOD PLASMA.

(Units are Sigma units per ml plasma).

Figure shown by median (50%)	Ranges		
	80%	10% lower	10% upper
7.6 (n=94)	3–17	0–2.9	17.1–32

per ml is somewhat wider than that given for normal human sera, namely 0-12 Sigma units per ml [8].

Borderline levels of activity in human sera are given as 12-20 Sigma units per ml [8]. It is of interest to note that these borderline values are all above the upper limit of the stated normal range. Figures 1 and 2 show that the same holds for the sheep, the upper 10% limit being 17.1-32 Sigma units per ml. Values of higher than 32 units per ml for sheep plasma must be considered as almost certainly abnormally elevated. This is somewhat higher than the corresponding figure of 20 units per ml given for human plasma [8]. The effects of muscular exertion, such as struggling during the taking of blood samples, on the plasma levels of this enzyme in the sheep have not been determined. It is inevitable that a certain amount of struggling occurs during this operation in practice and any variations due to this occurrence will thus have been incorporated in the 10% upper limit of the normal range. Such variations could conceivably extend this limit considerably beyond that found for human patients.

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THE ANTHELMINTIC EFFICACY OF PYRANTEL TARTRATE*

P. J. S. ANDERSON**

SUMMARY

Pyrantel tartrate at 20 mg/kg was highly effective against immature and adult *H. contortus, G. pachyscelis, N. spathiger, O. circumcincta, O. trifurcata, T. colubriformis* and *C. ovina*. Although it was highly effective against adult *O. columbianum* it was ineffective against immature *O. columbianum* even at dosages of 75 mg/kg.

INTRODUCTION

The recently synthesized compound pyrantel tartrate, trans 1-methyl-2 [2-(a-thienylvinyl)] 1, 4, 5, 6-tetrahydropyrimidine tartrate has been shown to be effective against a wide range of gastro-intestinal nematodes of sheep [1-5].

To estimate the lowest effective dose against most worm species sheep were dosed at 20 mg/kg. It is known that this compound is less effective against immature *Oesophagostomum columbianum* [5]. Consequently doses of 35, 50 and 75 mg/kg were used in trial against immature worms of this species.

EXPERIMENT I

Materials and Methods

Forty weaned Dorper (Dorset Horn X Black Head Persian) lambs, born, raised and maintained worm-free were divided at random into three groups, infested, treated and slaughtered as indicated in tables 1, 2 and 3.

Gaigeria pachyscelis larvae were placed on the skin of the rump in the first infestation, thereafter on the back of the head. (Table 1).

Methods of infestation of other species have been described [6].

Pyrantel tartrate was mixed with 20 ml of water and injected intra-ruminally in all experiments.

Procedures at autopsy have been described [7, 8].

Table 1—EXPERIMENT I — SECTION A; EXPERIMENTAL DESIGN

	No. of infective larvae dosed to each sheep.		
Day	G. pachyscelis	H. contortus	N. spathiger
−21	—	309	420
−16	498	487	431
−11	—	450	374
− 9	507	450	385
− 7	—	453	400
− 5	502	908	690
− 3	—	900	800
− 1	488	890	800
Total	1995	4847	4300
0	Treated Sheep 2 to 7 inclusive. Slaughtered Day 0 control Sheep 1.		
+ 4	Slaughtered Sheep 2 to 4 inclusive.		
+ 5	Slaughtered Sheep 8 to 11 inclusive.		
+ 6	Slaughtered Sheep 5 to 7 inclusive.		

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Table 2—EXPERIMENT I — SECTION B; EXPERIMENTAL DESIGN

Day	No. of infective larvae of **O. columbianum** dosed to each sheep.
−35	83
−30	100
−25	65
−20	84
−14	79
−11	80
− 9	77
− 7	81
− 5	98
− 3	102
− 1	103
Total	952

+ 0	Treated Sheep 13 to 16 inclusive at 75 mg/kg Treated Sheep 17 to 20 inclusive at 50 mg/kg Treated Sheep 21 to 24 inclusive at 35 mg/kg Slaughtered Day 0 control, Sheep 12
+ 8	Slaughtered Sheep 13 to 16 inclusive
+ 9	Slaughtered Sheep 17 to 20 inclusive
+10	Slaughtered Sheep 25 to 28 inclusive
+11	Slaughtered Sheep 21 to 24 inclusive

Table 3—EXPERIMENT I — SECTION C; EXPERIMENTAL DESIGN

	No. of infective larvae dosed to each sheep		
Day	C. ovina	Ostertagia spp.*	T. colubriformis
−73	35	—	—
−49	159	—	—
−36	205	—	—
−25	188	448	407
−20	188	488	403
−15	202	474	254
−11	198	480	401
− 9	191	188	344
− 7	221	341	223
− 5	324	392	776
− 3	264	1030	685
− 1	364	1030	685
Total	2539	4871	4178

0	Treated Sheep 30 to 35 inclusive. Slaughtered Day 0 control Sheep 29.
+ 4	Slaughtered Sheep 36 to 39 inclusive.
+ 5	Slaughtered Sheep 30 to 32 inclusive.
+ 6	Slaughtered Sheep 33 to 35 inclusive.

O. trifurcata** and **O. circumcincta.

With a few exceptions, in which two fifths of a specimen were examined, total worm counts were carried out.

The specimen was thoroughly stirred to obtain a representative sample and the first 50-100 worms recovered retained for differential identification. Larval stages were identified according to Veglia [9, 10], Ortlepp [11], Kates & Turner [12], Douvres [13, 14] and Threlkeld [15], and classified in their various stages according to Reinecke [16].

Results

Worms recovered are recorded in Tables 4, 5 & 6. The Day O controls were not included in the calculation of the average number of worms recovered from the controls.

Section A:

Haemonchus contortus, Nematodirus spathiger and *G. pachyscelis* (Table 4).

All the desired developmental stages of these worms were present at the time of treatment as shown by the Day O control (Sheep 1). Very uniform worm burdens had been established in the Day + 5 controls (Sheep 8 to 11).

With the exception of 103 adult *H.contortus* (Sheep 5) and 344 fourth stage larvae of *N.spathiger* (Sheep 4) very few worms were recovered from the treated sheep.

At 20 mg/kg pyrantel tartrate was more than 90 percent effective against all stages of *H.contortus, N.spathiger* and fourth stage *G. pachyscelis.*

Section B:

Oesophagostomum columbianum (Table 5).

The first two doses of infective larvae, checked for motility at the time of infestation, were apparently not capable of developing to adult worms as only eight adults were recovered from one control (Sheep 28). Subsequent doses of larvae developed to the fourth stage and were recovered in markedly uniform numbers both in the controls and treated sheep, indicating that the compound had no effect on fourth stage larvae.

TABLE 4—EXPERIMENT 1 — SECTION A; WORMS RECOVERED AT AUTOPSY

SPECIES	Stage \ Sheep No.	CONTROLS DAY 0	CONTROLS DAY + 5				Average	TREATED DAY + 4			TREATED DAY + 6			Average	Average Reduction
		1	8	9	10	11		2	3	4	5	6	7		
H. contortus	THIRD	80	0	0	0	0	0	0	0	0	0	0	0	0	—
	FOURTH	550	767	1176	1020	730	923	0	0	3	2	0	1	1	99.9%
	FIFTH	347	571	230	329	457	397	2	2	3	11	8	1	5	98.7%
	ADULT	186	629	508	473	595	551	10	0	1	103	5	2	20	96.3%
	TOTAL	1163	1967	1914	1822	1782	1871	12	2	7	116	13	4	26	98.6%
N. spathiger	THIRD	277	0	0	0	0	0	0	0	0	0	0	0	0	—
	FOURTH	472	1555	1249	843	1477	1281	10	6	344	35	68	58	87	93.2%
	FIFTH	53	333	183	152	216	221	0	1	2	0	6	1	2	99.1%
	ADULT	28	236	172	172	84	166	0	0	7	10	4	29	8	95.2%
	TOTAL	830	2124	1604	1167	1777	1668	10	7	353	45	78	88	97	94.2%
G. pachyscelis	THIRD	7	0	0	0	0	0	1	0	0	0	1	0	0	—
	FOURTH	179	210	169	191	253	206	0	0	0	1	1	0	0	99.8%
	FIFTH	0	0	0	0	0	0	0	0	0	0	0	0	0	—
	ADULT	0	0	0	0	0	0	0	0	0	0	0	0	0	—
	TOTAL	186	210	169	191	253	206	1	0	0	1	2	0	1	99.5%

Table 5— EXPERIMENT I — SECTION B; **O. COLUMBIANUM** RECOVERED AT AUTOPSY

Stage \ Sheep No.	CONTROLS DAY 0	DAY + 10				
	12	25	26	27	28	Av.
THIRD	33	0	0	0	0	0
FOURTH	153	71	46	37	112	67
FIFTH	18	0	2	0	7	2
ADULT	0	0	0	0	8	2
TOTAL	204	71	48	37	127	71

TREATED	DAY + 8				
75 mg/kg					
Stage \ Sheep No.	13	14	15	16	Av.
THIRD	0	0	0	0	0
FOURTH	90	110	85	178	116
FIFTH	4	0	0	0	0
ADULT	0	0	0	0	1
TOTAL	94	110	85	178	117

TREATED	DAY + 9				
50 mg/kg					
Stage \ Sheep No.	17	18	19	20	Av.
THIRD	0	0	0	0	0
FOURTH	279	56	112	75	131
FIFTH	10	0	0	0	2
ADULT	0	0	0	0	0
TOTAL	289	56	112	75	133

TREATED	DAY + 11				
35 mg/kg					
Stage \ Sheep No.	21	22	23	24	Av.
THIRD	0	0	0	0	0
FOURTH	77	176	118	41	103
FIFTH	0	20	4	1	6
ADULT	0	0	0	0	0
TOTAL	77	196	122	42	109

Section C:

Ostertagia spp., Trichostrongylus colubriformis and *Chabertia ovina* (Table 6).

All stages of development were present at the time of treatment (Day O control Sheep 29). With the exception of some variation in the numbers of fifth stage and adult *C. ovina*, the worm burdens in the Day + 4 controls were very uniform (Sheep 36 to 39).

Pyrantel tartrate was highly effective against all stages of *Ostertagia* spp. and *C. ovina* but efficacy varied from 79.5 to 80 percent against *T. colubriformis*.

EXPERIMENT II

A modification of the method of Banks & Michel[17] was used to test pyrantel tartrate against *G. pachyscelis*.

Material and Methods (Table 7)

A single dose of 300 infective larvae of *G. pachyscelis* were placed on the skin of one lamb (Indicator) and twelve Dorper ewes.

Twenty-four days later the "*Indicator*" lamb was slaughtered and worms recovered from lungs and small intestine to confirm larval viability.

Three groups of three sheep were treated 24, 45 and 68 days after infestation. These nine treated sheep and three untreated controls were killed on Day 69 and the worms recovered and counted.

TABLE 6—EXPERIMENT I — SECTION C; WORMS RECOVERED AT AUTOPSY

SPECIES	Stage \ Sheep No.	CONTROLS DAY 0	DAY + 4				Average	TREATED DAY + 6			DAY + 5			Average	Average Reduction
		29	36	37	38	39		30	31	32	33	34	35		
O. circumcincta **O. trifurcata**	THIRD	290	—	—	—	—	—	—	—	—	—	—	—	—	—
	FOURTH	762	307	641	829	382	540	29	166	17	51	13	20	49	90.9%
	FIFTH	510	309	439	1934	885	666	21	61	23	81	41	75	50	92.5%
	ADULT	837	1277	793	729	246	761	6	24	17	50	23	75	33	95.7%
	TOTAL	2399	1893	1873	2592	1513	1967	56	251	57	182	77	170	132	93.3%
T. colubriformis	THIRD	115	—	—	—	—	—	—	—	—	—	—	—	—	—
	FOURTH	374	138	74	194	176	145	86	59	14	9	0	0	28	80.7%
	FIFTH	176	346	526	383	363	405	70	83	79	80	81	103	83	79.5%
	ADULT	301	509	438	347	234	382	53	71	82	67	107	126	84	78.0%
	TOTAL	966	993	1038	924	773	932	209	213	175	156	188	229	195	79.1%
C. ovina	THIRD	155	26	46	41	86	50	2	0	0	0	0	2	0	99.4%
	FOURTH	340	291	323	287	470	342	1	2	1	5	0	4	2	99.4%
	FIFTH	69	81	10	150	47	72	1	1	0	0	0	1	1	98.6%
	ADULT	23	161	3	139	24	82	0	0	0	0	0	0	0	100%
	TOTAL	587	559	382	617	627	546	4	3	3	5	0	5	3	99.4%

Table 7.—EXPERIMENT II; MODIFIED BANKS & MICHEL TEST. WORMS RECOVERED AT AUTOPSY AFTER A SINGLE DOSE OF 300 **G. PACHYSCELIS** ON DAY 0.

Sheep No.	Day of Treatment	Day of Slaughter	G. pachyscelis recovered after autopsy		
			Third	Fourth	Adult
40	CONTROL	+24	2	21	0
50	CONTROL	+69	0	0	34
51	CONTROL	+69	0	0	46
52	CONTROL	+69	0	0	35
41	+24	+69	—	0	0
42	+24	+69	—	0	0
43	+24	+69	—	0	0
44	+51	+69	—	0	0
45	+51	+69	—	0	0
46	+51	+69	—	0	0
47	+68	+69	—	0	0
48	+68	+69	—	0	0
49	+68	+69	—	0	0

RESULTS

Worm recoveries summarised in Table 7 indicate that adult and immature *G. pachyscelis* were completely eliminated by pyrantel tartrate at 20 mg/kg.

EXPERIMENT III

The absence of adult *O. columbianum* in experiment I made it necessary to conduct a critical trial on these parasites by the method of Hall & Foster [18].

Materials and Methods

One sheep from a naturally infested flock was slaughtered to ascertain the degree of adult *O. columbianum* infestation present. A further ten were selected, weighed, five treated with pyrantel tartrate at 25 mg/kg and the remaining five treated at 20 mg/kg.

Faecal collection bags were attached to the sheep immediately after treatment, replaced at indicated intervals, the collected faeces washed through a 44 mesh sieve, and the adult *O. columbianum* collected and counted.

The sheep were slaughtered 42 hours after treatment, and the adult *O. columbianum* still present in the caecum and colon collected and counted.

RESULTS

The moderate worm burdens of Sheep 56, 60 and 62 precluded any conclusions being drawn as to the anthelmintic efficacy in these animals.

The majority of worms were expelled within 12 to 24 hours of treatment, very few worms being recovered at autopsy.

Where the total worm burdens varied from 78 to 297, anthelmintic efficacy exceeded 91 percent.

DISCUSSION

Three methods of testing the efficacy of an anthelmintic were used in this anthelmintic trial.

In view of the difficulty in repeatedly infesting sheep with *G. pachyscelis* the method of Banks & Michel [17] was essential to assess the anthelmintic efficacy of pyrantel tartrate against this species.

Due to poor larval viability of the first two doses of infective larvae of *O. columbianum* in Section B of this trial, naturally infested sheep were used to determine the efficacy of pyrantel tartrate against adult worms of this species using the critical test of Hall & Foster [18].

Table 8—EXPERIMENT III; RESULTS OF A CRITICAL TEST ON **O. COLUMBIANUM** (Hall & Foster)

	Treatment	25 mg/kg					20 mg/kg				
	Sheep No. / Time (hrs.)	53	54	55	56	57	58	59	60	61	62
O. columbianum recovered from faeces	8	0	1	0	0	1	0	0	0	0	0
	12	0	106	54	0	248	33	8	0	21	No faeces
	16	35	53	58	2	38	35	41	1	135	0
	20	133	6	15	1	2	24	12	0	49	0
	24	6	3	11	0	1	11	4	0	10	2
	36	1	1	0	0	1	5	8	0	1	5
	40	6	1	1	1	0	2	1	0	1	0
	42	4	2	1	0	0	0	0	1	0	0
Individual Total		185	173	140	4	291	110	74	2	217	7
Group Total		793					410				
O. columbianum recovered at autopsy		15	1	0	0	6	2	4	1	0	3
Reduction		91.9%	99.4%	100%	—	97.9%	98.2%	94.6%	—	100%	—
Average reduction		97.2%					97.6%				

The greater part of the nematode spectrum investigated was subjected to the exacting larval anthelmintic test of Reineke [19]. The importance of uniform worm burdens in this method has been reported [20]. Reinecke & Anderson [5] were unable to establish satisfactory worm burdens of *N. spathiger*, but in the present trial uniform worm burdens of this species were obtained.

Approximately 75 percent of the *N. spathiger* in the control sheep was recovered from the first 7 cm of the small intestine, confirming the findings of Tetley [21]. In treated sheep, however, only 4 percent of the worms recovered was found in the first 7 cm, the rest of the worms, particularly the fourth stage larvae in Sheep 4, being present in the last part of the small intestine. The worms were still alive, as demonstrated by their ability to pass through the gauze screens.

The disproportionally large number of *H. contortus* found in the small intestine of Sheep 5 is possibly further evidence of abnormal siting of worms following treatment. In view of the observations of Reinecke *et al* [22], who found that dead *H. contortus* were subject to trypsin digestion in the small intestine, it is unlikely that these worms could have survived for five or six days after treatment in the small intestine. In this instance, it is possible that movement of worms took place immediately after slaughter of the animal and before the separate parts of the bowel could be ligated.

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TWO CANINE INTERSEXES

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SUMMARY

Two canine intersexes are described. One was a chromosomal and gonadal male with immature testes (removed surgically at the age of six months) and posessing uterus and vagina, *epididymes* and *ductus deferentes*, but no male accessory sex glands. The external genitalia were of intermediate character.

The other was a *testiculo-ovotesticular* hermaphrodite with predominantly Müllerian duct development and female external genitalia. It had a masculine genetic constitution according to cytological study of bone marrow, but was an XX-XY mosaic according to nuclear sexing of polymorphs and of buccal, uterine and gonadal tissues.

The cases are discussed in the light of existing literature on canine intersexes.

INTRODUCTION

A considerable number of canine intersexes has already been described, the majority of reports being restricted to a morphological description [1-15]. Lately some authors have attempted to identify the genetic sex by examining blood smears for drumsticks [16, 17] or tissues for Barr bodies [18, 19, 20]. Since chromosome determinations have become a routine procedure, investigators [20,21] have determined the karyotype in order to gain more knowledge about the nature of the condition. Fortunately, the sex chromosomes in the dog are easily recognised, as they are the only submetacentric ones present: the female possesses two long submetacentric X-chromosomes, whereas the male has a single X and a small submetacentric Y-chromosome (fig. 2). The somatic chromosomes are all acrocentric.

Two cases which merit attention have come to our notice.

MATERIAL AND METHODS

A clinically healthy Alsation Bull terrier, about 9 months old (case I) was referred to us by a private practitioner who had performed an oöphorectomy and partial hysterectomy to effect sterilization when the dog was about 6 months old. Leucocyte cultures as well as bone marrow biopsies were used for chromosome determinations according to standard methods.

Case II was a clinically healthy Schipperke about 6 months old, referred to the Surgery Department of this Faculty for sterilization by ovarohysterectomy and subsequently sent home. Only bone marrow biopsies were used for chromosome studies.

In both cases buccal mucosa smears were also prepared according to the method of Schultz [19] for cytological sexing, while blood smears for polymorph sexing were prepared and stained with 10% Giemsa solution.

OBSERVATIONS

Case I was a sturdy, well-proportioned and lively dog. Its dolichocephalic head resembled that of a Bull Terrier. It urinated in a squatting position, i.e. assuming the female attitude, when first seen one month after oöphorectomy and partial hysterectomy had been performed. An enlarged clitoris (fig. 1) about 1 cm diameter protruded for ± 1.5 cm from the rather wide-stretched vulvar orifice situated well down from the ischial arch. An os penis was present and quite well developed. The gonads, obtained at operation at the age of 6 months, were found to be immature testes with quite well-developed seminiferous tubules lined only by Sertoli cells and occasional large clear cells resembling early spermatogonia. Interstitial cells were numerous but small. Neither primordial nor developing follicles were

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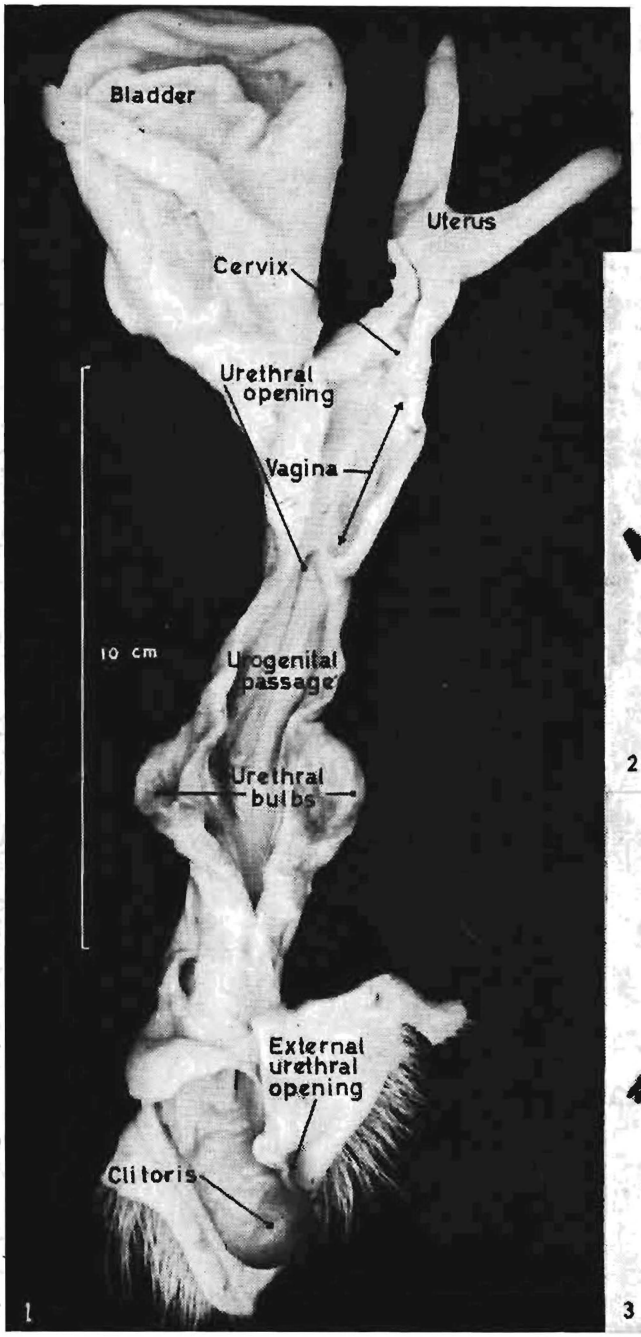
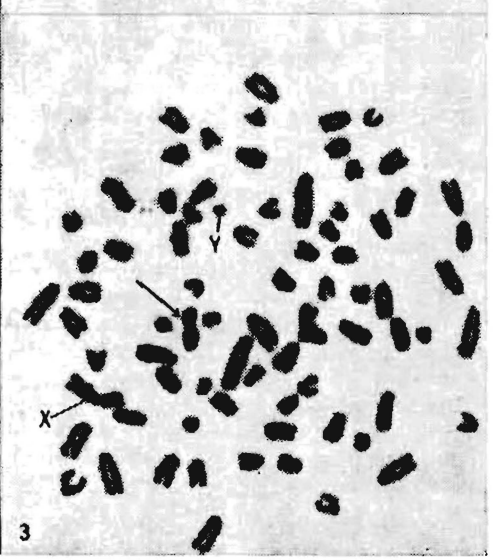


Fig.1 Urogenital passages of case I. An oophorectomy and a partial hysterectomy had been performed.



encountered, nor were any features reminiscent of ovarian structure.

Immature *epididymes* and *ductus deferentes* were present. Uterine horns, cervix and vagina were immature but distinct. The urethra, however, formed the main passage with the vagina leading from it as an accessory passage through a well-defined opening. Male accessory sex glands were completely absent.

No sex chromatin bodies were found in gonadal tissue and buccal smears. On the nuclei of the 1500 polymorphs counted, no drumsticks were found: a state typical for the male. Examination of a total of 100 metaphase spreads prepared from leucocyte cultures and bone marrow biopsies revealed a diploid number of 78 chromosomes with normal male sex chromosome configuration (fig. 2). In only one spread (fig. 3) was a metacentric autosome found. It had the normal number of 78 chromosomes.

Case II was a typical Schipperke of delicate build. The external genitals were female with the vulvar opening rather low down. The clitoris was enlarged; the *os penis* was absent. The bicornuate uterus was small, the endometrium possessing richly branched tubular glands. A fibrous *ligamentum teres uteri* with a central core of striated muscle connected each gonad to its respective inguinal ring.

In this case, too, the left gonad contained immature seminiferous tubules joined to a central *rete testis*. Interposed between them were numerous cordlike aggregations of Leydig cells. Some of the latter had large polyploid nuclei. Only Sertoli cells were present: spermatogenic cells were absent. The *tunica albuginea* was represented only by a wide, fibrous, rather acellular band without any sign of follicles or ovarian structure. Apart from the epididymal tubules which were lined by columnar epithelium with stereocilia no other male ducts or glands were seen. The left gonad thus was diagnosed as an immature *testis*.

The right gonad contained a number of fairly large follicles all with the granulosa layer represented by hypertrophied cells resembling granulosa lutein cells or even Leydig cells. No ova were found. On the one side immature seminiferous tubules with numerous interstitial cells were present. The tubules joined a central *rete testis*. No primordial follicles were encountered. The cortical region was fibrous and rather acellular. It was diagnosed as an *ovotestis*.

In contrast to the former case this animal had sex chromatin bodies present in the tissues of the uterus, *rete testes* and interstitial cells. Occasional Barr bodies were also encountered in the buccal smears, whereas eight drumsticks were found in 1100 leucocytes counted. Chromosome preparations from bone marrow biopsies revealed 46 excellent spreads all with the normal male karyotype.

This dog was diagnosed as a testiculo-ovotesticular hermaphrodite [22] with a masculine genetic sex. According to nuclear sex ing an XX-XY mosaic is indicated but this could not be confirmed by chromosome studies.

DISCUSSION

The morphology of the canine intersexes described to date tended to follow a definite pattern. Mostly both gonads were immature *testes* [1, 2, 3, 5, 6, 9, 10, 11, 19], the right one of which was either situated in the inguinal canal [2, 9, 11] or might even have descended and be present in the right *"labium"* [8, 11]. The testes were all immature and sterile, and in only one case [1] could the presence of spermatogonia be found. Consequently, the presence of spermatogonia in case I is rather exceptional. Occasionally both gonads have been reported as ovotestes [10, 15, 16] or the right [7] or left gonad [11,14] was an ovotestis and the contralateral gonad the testis. McFeely and Biggers [20] were the only authors thus far to have found a normal pair of ovaries in a canine hermaphrodite. Incidentally, this animal came on heat once every six months.

All authors have reported uterine horns and vagina to be present in a rather immature state but otherwise quite normal. Fallopian tubes were occasionally present [16] but mostly absent [10, 19]. In some intersexes with well-developed *testes* or *ovotestes, ductus epididymides* and/or *ductus deferentes* were present [1, 2, 3 5, 14, 16] where with exception of one case [20] slight evidence of a prostate was found; accessory glands have not been described.

Externally the dog intersexes had an abnormally large vulva with an enlarged clitoris varying in length from half an inch to almost two inches. The urethral opening usually was situated just dorsal to the clitoris. As a puppy the intersex dog appears normal — the clitoris enlarges only later [15] An *os penis* develops as a rule. Their sexual behaviour and manner of urination may resemble either of the two sexes. As a rule it appears, therefore, that in the development the gonads become hypoplastic testes or ovotestes; Wolffian duct development is suppressed and Müllerian duct development stimulated. Again McFeely and Biggers' [20] case is an exception to this rule, as it must be regarded as a gonadal female submitted to a masculinizing influence of unknown origin during prenatal development.

The urogenital sinus derivatives reveal an intermingling of male and female characteristics mostly with a hypoplastic penis. The *os penis* may be present or absent. If present, it has been regarded generally as direct evidence of androgen stimulation.

Besides the two cases described in this paper, the genetic sex has been determined in four cases only; three were genetic males [18, 19, 21], that described by McFeely and Biggers [20], by contrast, being a genetic female.

The animal described by King and Garvin [16] was a bilateral hermaphrodite with ovotestes and determined as a genetic female by identification of neutrophilic drumsticks in vessels in sections of uterus and ovotestes — aparently similar to the above genetic female [20] but with earlier or stronger masculinizing effect. Hence one can either have genetic females revealing varying degrees of masculinization or genetic males with varying degrees of feminization. Unfortunately, King and Garvin [16] do not mention whether an os *penis* was present or not in their animal but, judging from the other cases it appears that the presence or absence of an *os penis* may be a possible clinical feaure for differentiating between genetic males on the one hand and females or male/female mosaics on the other. It also appears that in the dog the genetic sex may coincide with the predominant gonadal sex, as is mostly the case in humans. Further evidence is still needed to substantiate or disprove both these hypotheses.

The dog intersex differs from the pig intersex [22]. The latter has always been found to be a genetic female apparently due to an early masculinizing influence. Recently, however, a pig intersex with an XX/XY leucocyte mosaicism but with only 10% male cells was described [23]. Such a finding raises the possibility that in pig chimaeras the male cells may have disappeared from the circulation of the intersex animal soon after birth, thus initiating the condition [22] but leaving no aetiological evidence. Such a possibility has still to be investigated. In case II nuclear sexing revealed a possible mosaic but this, unfortunately, could not be substantiated by chromosome determinations. Chimerism due to foetal chorionic anastomoses has not yet been described in the dog. It is extremely doubtful whether it could occur, as the morphology of the intersexual condition differs from that of the pig.

In case I only a single cell was found which contained an abnormal autosomal metacentric chromosome (fig. 3). It is homologous with one of the acrocentric autosomals and could have arisen as a translocation or inversion. It is also possible that this may be an artefact which could have arisen by twisting of the two chromatids around one another during preparation. On careful focussing however, it appeared that the chromatids were free from one another at both ends, i.e. that the constriction represented the site of the cenromere. As only one such spread was found it is extremely doubtful whether this chromosomal anomaly could be held responsible for the abnormality of the urogenital system. It is only mentioned here for record purposes and future reference.

ACKNOWLEDGEMENTS

Appreciation is expressed towards Dr. H. A. Davis of Johannesburg for bringing Case I to our notice and towards Mr. A. M. du Bruyn for the photographs. The Chief, Veterinary Research Institute, Onderstepoort, is thanked for permission to publish this article.

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A COMPARISON OF THE RATE OF REINFESTATION OF SHEEP WITH GASTRO-INTESTINAL PARASITES AFTER THE USE OF TWO DIFFERENT ANTHELMINTICS

S. STAMPA*, H. LINHART** AND R. SACHS***

SUMMARY

Worm counts 5-6 weeks after treatment with Neguvon A and Thiabendazole were compared with those of untreated controls. It is concluded that sheep dosed with Thiabendazole become more heavily reinfested with *Haemonchus contortus* than controls. This trend is not clear in the Neguvon A group. Post mortem counts of *Trichostrongylus* spp. were strikingly lower after the use of Thiabendazole.

Charting *Haemonchus contortus* against *Trichostrongylus* spp. at post mortem immediately after dosing shows that sheep dosed with Neguvon A tend to become heavily reinfested with *Trichostrongylus* spp. in preference to *Haemonchus contortus,* in contrast to sheep dosed with Thiabendazole, which become heavily reinfested with *Haemonchus contortus* in preference to *Trichostrongylus* spp.

It is considered that Neguvon A is preferable where and when *Haemonchus contortus* is of primary concern, and Thiabendazole is preferable where and when *Trichostrongylus* spp. are particularly important. Dosing with any one of the two drugs 3-4 weeks after use of the other is recommended where and when both parasites are of equal importance.

INTRODUCTION

In the course of extension work we repeatedly noticed heavy parasite burdens in sheep which had been dosed 4-6 weeks previously with drugs known to be highly effective against the species concerned. Repeated tests confirmed the full efficacy of these drugs [1]. It was thought possible that sheep treated with certain drugs become more heavily reinfested than those treated with others, or those left untreated.

Literature dealing with the influence of anthelmintic treatment on immunity to helminth infections has been reviewed by Soulsby [2]. Immunity depends on metabolic products of parasites living in the host, and acts (with few exceptions) in either of the following ways:

(a) The presence of nematode larvae in the third or fourth moulting stage causes a shedding of the adult worms of the same or other species.

(b) The presence of larvae or adult worms prevents development of larvae of the same species. Drugs highly effective against moulting larvae or against adults may interfere with these defence mechanisms, and domestic stock treated with such drugs can acquire heavy parasite burdens within a short time unless reinfestation is prevented.

Ross [3] found that Neguvon reduces immunity against *Haemonchus placei* in calves. Roberts & Keith [4] experienced the same with phenothiazine. Crofton [5] and Soulsby [2] found a reduced immunity against *Haemonchus contortus* and Dunsmore [6] a reduced resistance against *Ostertagia* after dosing with phenothiazine. Gibson [7] observed that treatment of infestation by adult *Trichonema* spp. with phenothiazine lowered the defence mechanism retarding larval development.

The opposite observation, i.e. drug treatments not interfering with immunity, were made by Banks & Mitton [8] who found no higher susceptibility to *Ostertagia* spp. in calves treated with Neguvon and by Leiper [9] who found that Thiabendazole did not increase but possibly reduced the susceptibility to *Ostertagia* spp., *Trichostrongylus* spp. and *Nematodirus* spp. in lambs.

The efficacy of Neguvon A against gastrointestinal parasites has been investigated by Behrenz [10], Knapp & Mosher [11], Meldal-Johnson, Muller & Thomas [12], and Stampa [1, 13]. Placing the main emphasis on results of

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critical and control tests, and evaluating the results of worm egg count trial with reservations (mainly when initial counts were low), the product can be accepted to remove gastro-intestinal parasites to the following extent at recommended dosing rates:

Haemonchus adults & 5th stage larvae: 100%.

Haemonchus 3rd & 4th stage larvae: 90-100% with the exception of the third moulting stage.

Ostertagia adults*: 50-60% irregular.

Trichostrongylus adults*: 70%.

Nematodirus adults*: 70% irregular.

Oesophagostomum adults*: 40% very irregular.

Trichuris adults*: 100%.

*The efficacy against immature stages of these spesies has not been investigated.

The efficacy of Thiabendazole has been tested by numerous investigators using modern techniques. With the exception of *Trichuris* spp., against which it is ineffective, the product can be regarded as practically 100% effective against all gastro-intestinal nematodes, including the immature stages. The efficacy against *Ostertagia* spp. is somewhat lower, perhaps 90%.

The aim of our investigations was to learn whether groups of sheep dewormed with Neguvon A or Thiabendazole become reinfested with gastro-intestinal nematodes at rates differing either from each other, or from untreated controls. Pasture management, such as moving stock to rested camps after dosing, was applied in the customary way on the respective farms.

MATERIALS AND METHODS

The trials were conducted on three farms, viz: Beaconsfield, Wildebeeskuil and Sea View. Merino wethers reared under natural conditions on the farms and of ages as stated below were used in all three trials: approximately 10-months old in the first trial, 8-10 months old in the second trial and 5-6 months old in the third trial. They were examined for worm eggs and placed into three or four groups each consisting of five animals with similar total egg counts. During the tests the animals were kept on the same type of pasture as that on which they had previously grazed in their former flocks.

Trial I.

At Beaconsfield, near Grahamstown, a camp of 108 acres consisted of an east-facing slope covered with fairly dense 'Fish River Bushveld', intermingled with patches of karoid bushes and different short grasses. The latter vegetation was rather dense along two narrow strips, one at the bottom and another at the top of the slope. Shrubs were absent from both these strips.

The ten test animals were returned to the same camp after being dosed, together with twelve lambs of the same flock dosed on the same day with Neguvon A. On this farm it was not possible to change camps after dosing.

The average rainfall of this area is 18-20 inches. The autumn season preceding the trial had brought good soaking rains. This was followed by 8 weeks of warm, fine weather, with very few showers. During the test period, three small showers of rain fell, and an average daily maximum temperature of 18.6° C and minimum of 5.4° C indicate that warm winter weather prevailed.

Trial 2.

Four irrigated grass clover paddocks of one acre each were available on Wildebeeskuil in the flat country of the Great Karoo, near Pearston. The fifteen experimental animals were the first stock introduced after the winter. Frost can be expected on at least 60 nights per year in this area, with temperatures as low as -7C. Each paddock was grazed for one week, thereafter irrigated and rested for three weeks. After being dosed, the test animals were moved to the adjoining paddock, together with 27 flockmates not included in the experiment. The latter were dosed on the same day with an experimental formulation effective against all species concerned, and then dosed again 3 weeks later with the same drug.

Detailed temperature records were not available. Warm summer weather prevailed throughout the test period. The average daily maximum and minimum temperature was estimated at 33° C and 15° C respectively. No rain fell.

Trial 3.

On Sea View, near the mouth of the Fish River, sheep were kept on sour grassveld with a few small patches of dense shrub. The fifteen experimental animals grazed with a flock of approximately 1,600 sheep and 120 cattle in a camp of 270 acres, and slept in kraals at night. Kraals and camp were changed after dosing. All sheep not included in the experiment were dosed on the same

day with Thiabendazole, and three weeks after commencement of the trial with Neguvon A.

Warm summer weather prevailed during the test period, broken several times by spells of cold, cloudy weather, with misty or soaking rain. Detailed temperature records are not available. During spells of fine weather, daily maxima and minima in this area reach 35° C and 15° C respectively. During rainy spells the corresponding temperatures are approximately 18° C and 12° C.

Neguvon A and Thiabendazole were supplied by the producers, Messrs. Agro-Chem. (Pty.) Ltd. and Messrs. Merck, Sharp & Dohme (Pty.) Ltd., respectively.

Faecal worm egg counts were carried out on a large number of animals on each farm: fifteen with similar total egg counts were selected for the slaughter trial (10 animals only at Beaconsfield). On each farm, each drug was administered to five animals. On Wildebeeskuil and Sea View five further animals were left untreated as controls. We included a further group of five animals treated with phenothiazine on each farm. The results of these groups show no consistent trend and, therefore, are not included.

The animals in each group were given the same dosage of active ingredient per kg liveweight i.e. 50 mg/kg Neguvon A (in the Sea View trial 35 mg/kg), and 50 mg/kg

Table 1—WORMS PER SHEEP AT AUTOPSY (AVERAGE PER GROUP OF 5 SHEEP) AND SIGNIFICANCE ACCORDING TO MANN-WHITNEY TEST[15].

	adults†			5th larvae			4th larvae		
	Neg.	Thia.	Contr.	Neg.	Thia.	Contr.	Neg.	Thia.	Contr.
HAEMONCHUS CONTORTUS									
Beaconsfield	2	419							
		xxx*							
Wildebeeskuil	158	215	40	84	190	30	220	372	154
		xxx			xx			xx	
	x								
Sea View	486	855	1194	108	249	287	357	430	992
		xx			xx			x	
								xx	
OSTERTAGIA SPP.									
Beaconsfield	318	479							
Wildebeeskuil	3404	2343	4440	696	866	622	990	745	1998
		x						x	
Sea View	596	601	552	90	160	175	274	230	642
TRICHOSTRONGYLUS SPP.									
Beaconsfield	12761	2506							
	x								
Wildebeeskuil	14276	5987	21573	687	450	712	583	528	982
	x								
		x							
Sea View	3494	1813	7648	268	340	605	267	324	400
	xx	xx			xx				
		xx							

Nematodirus spp., Oesophagostomum spp. and **Trichuris spp.** present in small numbers only. Trends neither uniform nor significant.

†on Beaconsfield, adults + 5th larvae.

Significance: xxx = $p < 0.01$, xx = $p < 0.05$, x = $p < 0.10$,
no sign = $p > 0.10$ = not significant.

Table 2—WORMS EXCRETED PER SHEEP (AVERAGE PER GROUP OF 5 SHEEP) AND SIGNIFICANCE OF DIFFERENCES ACCORDING TO MANN-WHITNEY[15].

	HAEMONCHUS CONTORTUS		OSTERTAGIA SPP.	
	Neguvon A	Thiabendazole	Neguvon A	Thiabendazole
Beaconsfield	40	393 (xx)	167	1287 (xxx)
Wildebeeskuil	0	0	1633	473 (xx)
Sea View	1143	880	133	257 (x)

	TRICHOSTRONGYLUS SPP.		OESOPHAGOSTOMUM SPP.	
Beaconsfield	4753	7273 (x)	2	10 (xx)
Wildebeeskuil	4940	7297	122	106
Sea View	1537	2020	0.2	2.4

	TRICHURIS SPP.	
Beaconsfield	9.8	0.2 (xx)
Wildebeeskuil	0.2	0
Sea View	0.8	0

Nematodirus spp. Trends neither uniform nor significant.

Significance: xxx = $p < 0.01$, xx = $p < 0.05$, x = $p < 0.10$, no sign = $p > 0.10$ = not significant.

Thiabendazole. Five ml of a 10 percent copper sulphate solution were given immediately before the Nevugon A as recommended by the manufacturers.

Faecal collection bags were attached to the dosed animals for 56 hours after treatment and their droppings examined for excreted worms. Egg counts and larval differentiations were done at weekly intervals after dosing until immediately before slaughter. Six weeks after dosing, (five weeks at Sea View, as some animals were in danger of dying from worm burdens) all 40 dosed and control animals were sacrificed and worms counted in ingesta and in the artificially digested gut. A sampling method as desribed by Reinecke[14] was employed for the small species, and total counts for the *Trichuris* and *Oesophagostomum* species were made.

RESULTS

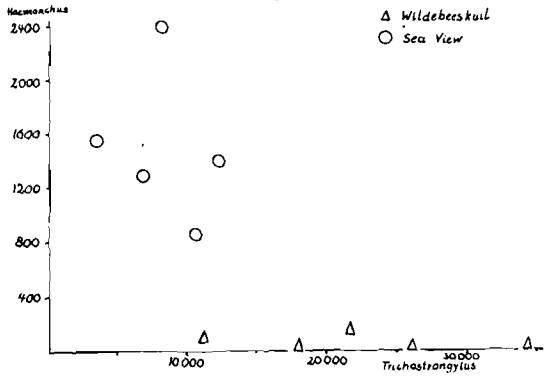
In the first instance we compared the number of worms found *post mortem* and summarised the results in Table 1. The number of worms excreted after dosing is given in Table 2.

The bivariate distribution of *Haemonchus* spp. and *Trichostrongylus* spp. in untreated controls on the farms Sea View and Wildebeeskuil is shown in Graph 1.

We made an effort to establish whether the higher initial efficacy of Thiabendazole against *Haemonchus* or other parasites was responsible for the higher rate of reinfestation with *Haemonchus* spp.

The correlation between *Ostertagia* spp. excreted and *Haemonchus* spp. found at *post mortem*, was inconsistent. The correlation between *Trichostrongylus* spp. excreted and *Haemonchus* spp. found at *post mortem* was consistently positive for the group totals.

Graph 1. *Distribution of Haemonchus and Trichostrongylus adults and 5th stage in control sheep*



Within each group this correlation however, was consistently negative. It is not useful, in our experience, to correlate the number of *Haemonchus* spp. excreted with any other figure, as a high and variable percentage of these parasites are digested after being killed by the drug.

DISCUSSION

The rate of reinfestation after the use of these potent anthelmintics was very high. We consider that the following worm burdens are pathogenic: 1,000 adults (including 5th stage) of *Haemonchus* spp., 4,000 *Ostertagia* spp. or 10,000 *Trichostrongylus* spp., and believe that combinations of smaller numbers of the different species represent a dangerous total. If this is correct, all stock in these trials carried pathogenic worm burdens 5-6 weeks after being dosed.

There may be an objection to sheep being returned to the infested camp after dosing at Beaconsfield, thereby increasing the danger of reinfestation. This procedure was purposely tested, as many farmers in the higher-rainfall regions cannot change camps each time after dosing. The reinfestation rate is, however, to be regarded as very high; the trial took place during a fairly dry winter when the activity of worms is at its lowest.

Camps were changed after dosing on the two other farms. The reinfestation by untreated controls was counteracted at the same time by introducing a fairly large number of treated sheep with the experimental flock. These steps met with no success in the case of *Haemonchus* spp. *Haemonchus* burdens of groups 5-6 weeks after treatment were similar or even higher than those of undosed controls. The success of the complete removal of all stages by dosing was practically lost within this very short period.

Considering the high efficacy of Thiabendazole against all stages of *Haemonchus* spp., we are inclined to accept that high *Haemonchus* counts at *post mortem* (significantly larger than those of controls at Wildebeeskuil, slightly and not significantly smaller than those of controls at Sea View), prove that the use of this drug interfered with immunity against this parasite.

Such a definite statement cannot be made for the other drug. Leiper's [9] observation of low reinfestation rates with *Trichostrongylus* spp. after the use of Thiabendazole is supported, although not significantly [50].

More *Haemonchus* were found at autopsy in the Thiabendazole groups than in the Neguvon A groups. This difference was only significant on the farm Beaconsfield, but its consistency in all observations for all stages of development convinces us that sheep become heavily reinfested with *Haemonchus* spp. more rapidly after dosing with Thiabendazole than after dosing with Neguvon A.

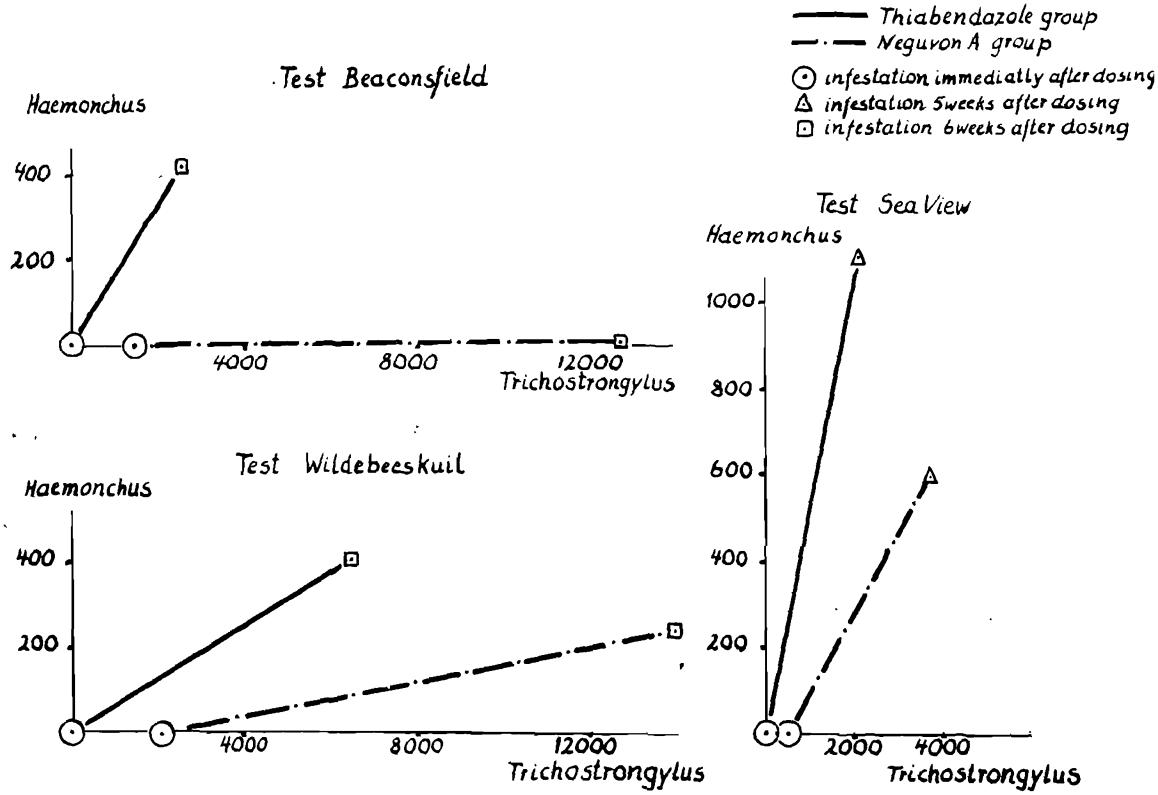
More *Trichostrongylus* spp. were found at *post mortem* in the Neguvon A groups than in the Thiabendazole groups, with the exception of 5th stage larvae at Sea View. For adults at Wildebeeskuil and Sea View, and for adults and 5th stage larvae at Beaconsfield, this tendency was significant, at the same time too large to be exclusively due to the lower initial efficacy of Neguvon A. There seems to be little doubt that sheep become reinfested with *Trichostrongylus* spp. faster after dosing with Neguvon A than after dosing with Thiabendazole.

The situation can probably be better understood by an examination of Graph 2. The average number of *Haemonchus* spp. and *Trichostrongylus* spp. present immediately after dosing has been connected in this graph by straight lines with the numbers present at autopsy. The connecting lines show the trend of reinfestation. Worms present immediately after dosing are calculated by the following equation:

$$p = \frac{\text{Worms present before dosing} \times (100 - \text{efficacy})}{100}$$

calculating the worms present before dosing from the pre-dosing egg count, or the number of worms excreted within 56 hours of dosing. The high efficacy of both drugs against *Haemonchus* spp. eliminates possible faults of this technique. We tested the correlation

Graph 2. Uptake of *H. contortus* and *Trichostrongylus* spp. after dosing. Neguvon A compared with Thiabendazole.



between *Trichostrongylus* spp. eggs per gram of faeces and adult worms present at *post mortem*, using all sheep, and found it to be significant. Both methods of calculating worms present before dosing, gave similar results, thereby justifying the procedure.

Graph 2 shows the tendency of sheep dosed with Thiabendazole to become heavily reinfested with *Haemonchus* spp. in preference to *Trichostrongylus* spp. whereas sheep dosed with Neguvon A become heavily reinfested with *Trichostrongylus* spp. from the same environment, in preference to *Haemonchus* spp. The tendency expressed by the tangent of the angles is consistent and highly significant: Beaconsfield $P < 0.01$; Wildebeeskuil $P < 0.05$; Sea View $P < 0.01$.

Graph 1 shows a negative correlation between *Haemonchus* spp. and *Trichostrongylus* spp; sheep which harboured many *Haemonchus* spp. had few *Trichostrongylus* spp. and vice versa. With which of the two species sheep become reinfested by preference, may depend on the availability of infective larvae[5]. In contrast with that tendency, sheep treated with Neguvon A consistently become reinfested with *Trichostrongylus* spp. in preference to *Haemonchus* spp. Conversely, sheep treated with Thiabendazole become reinfested with *Haemonchus* spp. in preference to *Trichostrongylus* spp. This observation is based on statistically highly significant evidence, and is likely to be a basic rule.

The consistent negative correlation between *Trichostrongylus* spp. excreted and *Haemonchus* spp. found at post mortem within each group, induces us to reject the validity of a positive correlation between the Thiabendazole and Neguvon A group totals.

It cannot be accepted as proved that the initial efficacy against *Trichostrongylus* spp. causes a high rate of reinfestation with *Haemonchus*.

We remain uncertain as to the reason for the selective reinfestation after the use of Neguvon A and Thiabendazole. Soulsby [2] suggests that the high efficacy of a drug against immature stages may interfere with the host's immunity which depends on the presence of such living larvae. The negative correlation between adult *Haemonchus* spp. and *Trichostrongylus* spp. at *post mortem* points to the possibility that a drug highly effective against *Trichostrongylus* spp. may reduce the *Trichostrongylus* adult's resistance to *Haemonchus* adults. Reinfestation with a large number of *Haemonchus* spp. may in turn lead to a reduced reinfestation with *Trichostrongylus* spp. Until this question is clarified, it must be regarded (for reasons unknown) that it is an intrinsic characteristic of Neguvon A to cause slower reinfestation with *Haemonchus contortus* and more rapid reinfestation with *Trichostrongylus* spp., as compared with Thiabendazole.

RECOMMENDATION AND CONCLUSION

In localities and seasons of high *Haemonchus* spp., activity, the use of Neguvon A for deworming is preferable to Thiabendazole, because the high initial efficacy of the former is combined with a low rate of reinfestation. Conversely, in localities and seasons of high *Trichostrongylus* spp. activity, Thiabendazole is preferred for the same reasons.

Considering the high rate of reinfestation under conditions typical of normal South African farm management, alternating these two drugs is recommended where both species of parasite are present in pathogenic numbers. Under such conditions, lambs need to be dosed at intervals shorter than 5 weeks.

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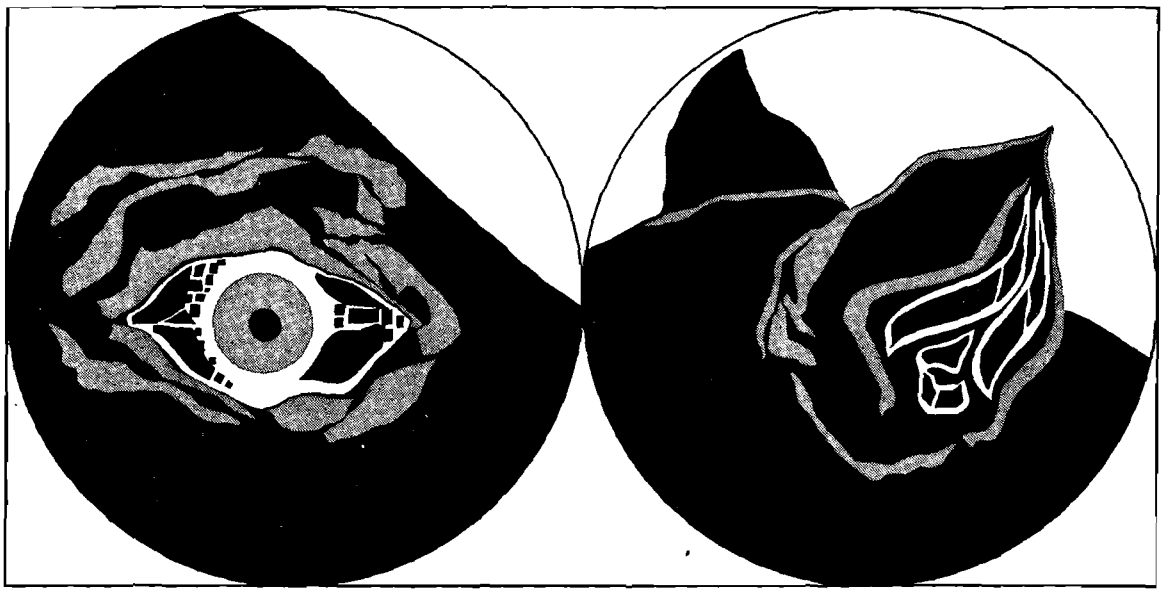
In superficial eye and ear conditions of domestic animals...



Neo-Cortef with Tetracaine
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Jl S. Afr. vet. med. Ass. 39 (1): 69—72 1968

A HYMENOLEPID CYSTICERCOID FROM THE LIVER OF CHINCHILLA

J. L. DU PLESSIS AND MARIE COLLINS*

SUMMARY

Cysticercoids, probably those of *Hymenolepis nana fraterna,* were observed in the liver parenchyme as well as in branches of portal vein of a chinchilla as seen on section.

INTRODUCTION

The formalin-fixed liver of a chinchilla, *Chinchilla laniger* Molina, 1782, was found to contain hymenolepid cysticercoids. The only other record of such cysticercoids in a liver concerns *Hymenolepis nana fraterna* (Stiles, 1906) in *Cryptomys darlingi* Thomas, 1895 [1]. These cysticercoids are usually confined to the intestinal mucosa [2-6].

Coenurus serialis, the larval stage of *Taenia serialis* (Gervais, 1847) Baillet, 1863, is a common and widespread parasite of chinchilla in the United States of America [7] and in South Africa [8]. A single case of *Cysticercus pisiformis,* the larval stage of *Taenia pisiformis* (Bloch, 1780) Gmelin, 1790, has been found in the liver of a chinchilla [9].

MATERIALS AND METHODS

Routine histological sections were cut. Cysticercoids were dissected from the liver substance and scolices mounted in Berlese mounting fluid. Rostellar hooks were measured as described by Meggitt [10].

RESULTS

Rostellar Hooks

The rostellar hooks vary from 22 to 36 in number and from 9.9 μ to 15.8 μ in length (Fig. 2D).

Histopathology

Cysticercoids were found in all the sections examined. They were generally in the hepatic tissue away from the portal triads, (Figs. 1 A and C, 2 F), but several sections of the parasite were also observed in the branches of the portal vein (Fig. 1B). Other histological features were cellular infiltration around the parasites by polymorphonuclear eosinophils (Figs. 1C, 2F), parasite tracts characterised by focal necrosis and haemorrhage, (Fig 2C) and marked fatty degeneration (Fig. 1B).

DISCUSSION

Table—NUMBER AND SIZE (in μ) OF ROSTELLAR HOOKS OF **H. NANA FRATERNA**

Author	Number	Size
Dujardin (1945)[11]	20—24	15—17
Joyeux (1920)[3]	22—24	21.3—24
Megitt and Subramanjan (1927)[12]	20—30	14—18
Mahon (1954)[1]	21—22	18—19
	23*	16.1—19.6*
These data	22—36*	9.9—15.8*

*Recorded from cysticeroids.

This material differs in the number and the size of the rostellar hooks from that of *Hymenolepis nana fraterna.* These differences may be due to immaturity of the cysticercoid, as Scott [13] showed that the rostellar hooks of *Hymenolepis longior* Baylis, 1923 (synonym: *H. nana fraterna)* increase in size from 15 to 22 μ between the fourth and seventh day.

The adult of *Hymenolepis microstoma* (Dujardin, 1854) parasitizes rodents and is more often found in the bile ducts than the duodenum; but Dvorak et al [14] showed that an invertebrate host is indispensible to this cestode, which has not yet been recorded from chinchilla. It is therefore unlikely that the cysticercoids described above belong to this species.

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Although it is not possible to determine the species of the cysticercoids in this case they are probably those of *H. nana fraterna,* which is known to parasitize chinchilla in the United States [15] and in South Africa [16]. Adult *H. nana fraterna* recorded from chinchilla in South Africa have 22 rostellar hooks, 15.1 to 16.8 μ long, [17] which are of similar shape to those described by Joyeux [3] and Mahon [1].

Intestinal infestation of chinchilla with *H. nana fraterna* is characterised by the presence of cysticercoids in the lamina propria and in the submucosa of the small intestine (Fig. 1D and 2E). It is possible in the case reported here that cysticercoids may have gained the portal system via the mesenteric veins.

Mahon [1] states that the cysticercoids in the *C. darlingi* liver were found in the bile ducts, but the photomicrograph (plate I, fig. 2) produced by this author demonstrates the parasite in a branch of the portal vein situated between the normal unparasitized bile ducts. The bile ducts in the chinchilla likewise were not affected.

ACKNOWLEDGEMENTS

The authors thank the Chief, Veterinary Research Institute, Onderstepoort, for permission to publish this report, Mr. G. Claussen, Somerset West, and the Officer in Charge, Veterinary Investigation Centre, Stellenbosch, for submitting the specimen.

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Fig. 1: (p. 70)

A. Section through the scolex of a cysticercoid in the liver , demonstrating the hooks. H and E, X 200.
B. Section through a cysticercoid in a portal vein of the liver. Severe fatty degeneration of hepatocytes. H and E, x 75.
C. Section through cysticercoid in liver, surrounded by eosinophils. H and E. X 200.
D. Small-intestine with cysticercoid in lamina propria. H and E, X 200.

Fig. 2: (p. 72)

A. Unstained preparation of scolex with rostellum in centre, surrounded by suckers. Phase-contrast, X 480.
B. Three isolated cysticercoids, unstained, X 30.
C. Necroses and haemorrhage in adjacent liver lobules H and E, x 75.
D. Variation in shape of hooks. Photomicrographs.
E. Cysticercoid in submucosa of small-intestine. H and E, X 200.
F. Eosinophils and round cells surrounding parasits (arrow), in liver. H and E, X 200.

Jl S. Afr. vet. med. Ass. 39 (1): 73—75 1968

A COMPARATIVE STUDY ON THE LIFESPAN OF ERYTHROCYTES AND OTHER HAEMATOLOGICAL DATA IN RECOVERED CASES OF GEELDIKKOP AND CLINICALLY NORMAL SHEEP

L. P. NEETHLING*, J. M. M. BROWN** AND P. J. DE WET**

SUMMARY

Recovered cases of geeldikkop show a persistent moderate to severe hypochromic microcytic (or normocytic) anaemia up to four months after the disappearance of the acute manifestations of the disease. This is associated with a decreased half-life time and increased mass of the erythrocytes and a mild hypoproteinaemic state. There is also an absolute elevation of α 2-globulins for which possible reasons are advanced. The data presented are regarded as further evidence of the operation of a haemolytic state in the pathogenesis of geeldikkop.

INTRODUCTION

The existence of a haemolytic syndrome in geeldikkop has been postulated earlier[1]. The life span of the erythrocytes has been determined in geeldikkop cases. If it is shortened, the presence of an haemolytic disease is indicated. Hitherto only recently recovered cases of the disease have been studied. In this paper results are presented of studies performed on animals four months after recovery from the acute manifestations of geeldikkop.

MATERIALS AND METHODS

Twenty four adult Merino ewes and wethers which had had geeldikkop during January and February of 1967 were used for these studies, which commenced during June of the same year and terminated three months later. As control animals, we used twenty four adult Merino ewes and wethers drawn from the pool of available animals at Onderstepoort. These sheep had been housed here for at least two years. All the animals were given lucerne hay, teff hay, and water *ad libitum*.

Haematological studies were undertaken using standard procedures. Heparin was used throughout as anticoagulant. Total plasma proteins were determined by the method of Weichselbaum[2] and protein fractions by microzone electrophoresis as described by Van Zyl[3]. Autologous red cells were labelled with Cr 51 as outlined by Mollison and Veall[4]. Sterile isotonic sodium chromate with a specific activity greater than 20 mC/mg was used. Ten ml of blood was centrifuged, the cells removed, washed once with 15 ml ACD solution (trisodium citrate, 3.0g; sodium dihydrogen phosphate ($2H_2O$), 0.015g; glucose, 0.2g; water to make 100 ml; pH 6.9, without autoclaving).

Excess ACD was removed and 100 μC of Cr51 was added with slight shaking. The cells were incubated at 37°C for 30 minutes after which they were washed twice with 2% w/v autologous plasma in 0.9% NaCl solution. They were then made up to 10 ml with the plasma-saline solution. A known amount of this solution in the range 7-8 ml was injected intravenously into each experimental animal. The one hundred percent reading was made on a 5 ml sample collected 15 minutes after the injection and this value was used in computing red cell volumes. Exactly one ml of the labelled red cells served as standard. Counting was performed with a Philips Automatic Well-type Scintillation Detector (Type PW 4003) with a $1\frac{3}{4}$ x 2" NaI/TI crystal.

All subsequent analyses were performed on 5 ml blood samples collected at suitable intervals over a period of at least sixty days.

It was ensured that all counts registered fell in the 95% confidence interval. Data were analysed statistically using standard procedures.

RESULTS

These are presented in the table. Red cell counts and packed cell volumes were also done on each blood sample. These re-

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Table.—HAEMATOLOGICAL DATA FROM NORMAL AND RECOVERED GEELDIKKOP CASES

Determination	Normal Sheep			Geeldikkop Cases			Statistical* Evaluation
	n	Mean Value	SD	n	Mean Value	SD	
Haemoglobin (g%)	20	8.31	0.20	20	7.03	1.08	Highly significant
Mean Corpuscular Haemoglobin (MCH = γγ)	20	8.45	0.72	20	6.62	0.87	Highly significant
Mean Corpuscular Haemoglobin Concentration (MCHC = %)	20	23.6	0.54	20	19.5	1.80	Highly significant
Mean Corpuscular Volume (MCV = Cμ)	20	35.9	3.0	20	34.0	3.6	Almost significant
Total Plasma Proteins (g%)	24	7.54	0.42	24	6.86	0.71	Highly significant
Plasma Albumins (% of total)	24	48.59	3.76	24	47.87	4.25	Highly significant
Plasma α 2-globulins (% of total)	24	5.68	0.72	24	5.63	1.27	Non-significant
Plasma α 1-globulins (% of total)	24	9.75	0.98	24	10.67	1.46	Significant
Plasma β-globulins (% of total)	24	6.72	1.09	24	6.77	1.26	Non-significant
Plasma γ-globulins (% of total)	24	22.73	3.92	24	21.29	3.92	Non-significant
Plasma γ-trailing fraction (% of total)	24	6.53	2.81	24	7.78	2.47	Non-significant
Red Cell Volume (ml red cells/Kg bodyweight)	18	19.6	2.7	19	24.4	6.5	Highly significant
Apparent halflife of erythrocytes (days)	18	15.0	0.2	19	12.9	2.1	Highly significant

n = number of samples; *SD = Standard Deviation.*

*If P is < 0.05 the difference is non-significant. If 0.01 < P < 0.05 the difference is almost significant. If 0.001 < P > 0.01 it is significant and if P < 0.001 it is highly significant, using Student's T-test.

sults are not shown in this table but since they were used for calculation of the absolute indices they are incorporated into these data.

DISCUSSION

It has been demonstrated that a feature of the acute manifestations of geeldikkop is a **hypocythaemic hypochromic macrocytic** anaemia of more or less severity depending upon the stage of the disease [1]. Such anaemias are usually encountered during the course of haemolytic diseases [5, 6, 7]. The anaemic episodes of enzootic icterus are typically **hypocythaemic normocytic normochromic** or **hypocythaemic macrocytic hyperchromic** (or **normochromic**) [1] in nature.

The data presented in the table show that the anaemia of typical geeldikkop cases persists in recovered animals as a **hypochromic microcytic** (or **normocytic**) type for as long as four months after the disappearance of the acute manifestations and possibly longer. This anaemia is associated with a shortening of red cell half-life time and hence of red cell life span which is a well known phenomenon in recurrent haemolytic states of man and animals. It is also apparent from the data in the table that the anaemia of recovered geeldikkop cases is associated with an increased total red cell mass. This is most likely the result of a physiological overcompensation in response to the demands made on the sheep's body by the continuous low grade haemolysis. The erythrocytes thrust into the systemic blood circulation as a result of this stress are known to have a markedly increased fragility and are largely immature [1]. This later finding is in complete agreement with the shortened red cell half-life now demonstrated.

The data presented also demonstrate that recovered cases of geeldikkop are mildly hypoproteinaemic when compared with the control animals. It would thus appear that there is a decrease in plasma albumin levels in the recovered geeldikkop cases. If, however, the figures for the "γ-trailing fraction" are disregarded (of unknown composition or significance [3]) then the ratio of total plasma proteins to albumins in both groups of animals is identical (1.91 as against 1.93). This indicates no change in the albumin fraction. The only difference of significance is therefore the increase in the α2-globulin fraction in the plasma of the recovered geeldikkop sheep. Considering the decrease in total proteins, this constitutes an 18% elevation of plasma α2-globulins. Little is known of the functions of this plasma protein fraction. It is known that this fraction embraces heat stable inhibitors of viral haemagglutination. The levels of these inhibitors rise markedly in a number of diseases involving inflammatory processes which is consistent with the gross pathology of geeldikkop. The major selenium bearing proteins in human plasma are the α-and β-globulins and not any other protein fractions [8]. Incorporation of selenium into the members of this fraction will undoubtedly change their effective functions as a result of stereospecific changes in their structure. This in turn could lead to: (a) a decreased effectivity in anti-inflammatory action or (b) a lowering of the inhibition of haemagglutination. The net result must surely be a compensatory increased rate of α2-globulin synthesis.

ACKNOWLEDGEMENTS

The Chief, Veterinary Research Institute, Onderstepoort is thanked for permission to publish this paper.

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ARMILLIFER ARMILLATUS (WYMAN) (ORDER: PENTASTOMIDA) FROM SLAUGHTER STOCK

R. DU TOIT* AND R. J. SUTHERLAND**

SUMMARY

Two cases of infestation of bovines by the immature stages of the pentastomid, *Armillifer armillatus* in adult bovines are recorded for the first time. Illustrations of the adult and immature forms are provided.

INTRODUCTION

Next to *Linguatula*, the "tongue worm" of carnivores and the only member of this interesting order of which the adults occur in mammals, *Armillifer armillatus* is probably the most widely distributed species of the Ethiopian region, the adults occurring in the respiratory passages of a large number of snakes, the African Python constituting the most common host [1].

The immature stages have been recovered from wild **Primates, Insectivora, Carnivora, Tubuldentata, Artiodactyla, Rodentia** and records exist of nymphs from man in South Africa and Nigeria.

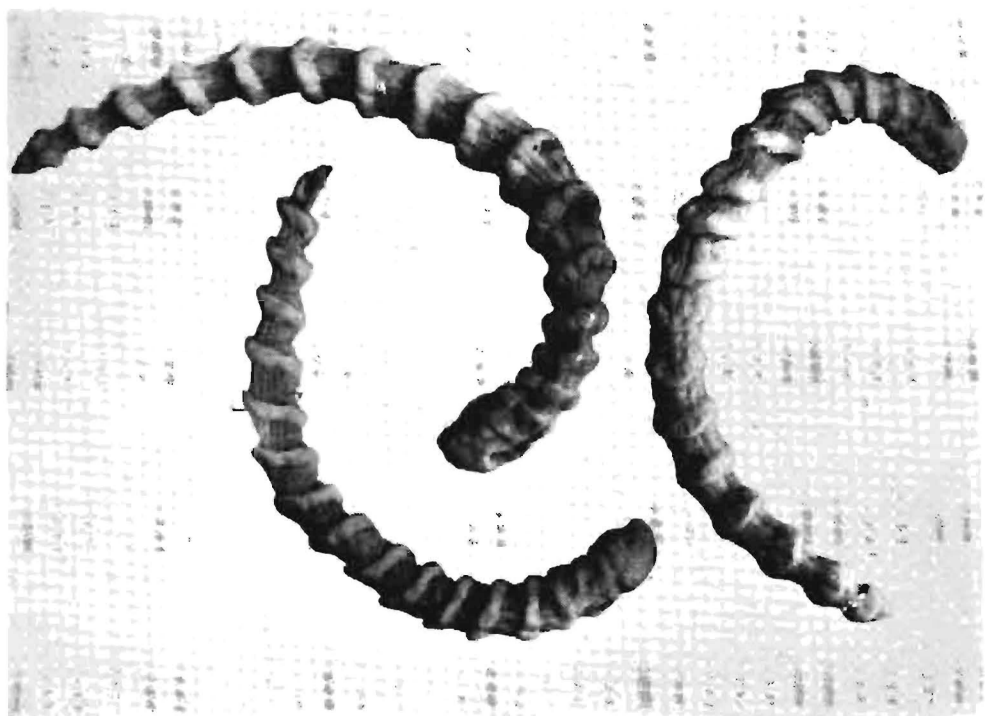

Fig. 1.
Adult **Armillifer armillatus** X 1

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** Manzini, Swaziland.

The embryonated eggs passed by the adults (fig. 1) contaminate pasture and on being ingested accidentally by almost any species of mammal are capable of further development in such intermediate hosts where encystment in various organs occurs, a favoured site being the serous membranes of the body.

True intermediate hosts are most commonly found among various species of monkeys which form a common source of prey for the python. Upon digestion the nymph escapes and by a route which has as yet not been determined, attaches itself to the mucosa of the respiratory passages of the snake where development to the adult stage occurs.

MATERIAL AND METHODS

During the course of routine inspection of beef carcases intended for export at the export abattoir at Matsapa, near Manzini, Swaziland, during July 1967, one of us (R. J. S.) encountered a large number of spirally coiled wormlike parasites showing circular thickenings of the integument and firmly adherent to the peritoneum of a mature steer. Several areas two to three inches in diameter situated towards the dorsal surface of the abdomen each contained about a dozen of these organisms (fig. 2). On closer inspection the parasites were found to be provided with two pairs of fang-like hooks surrounding a circular oral aperture at the anteroventral aspect of the anterior end of the body. On removal they displayed some sluggish non-directional movement.

Preserved portions of the peritoneum were forwarded to Onderstepoort where a tentative identification of immature *Armillifer armillatus* was made. To confirm this the material was forwarded to Dr. F. Zumpt of the S.A. Institute for Medical Research who in turn forwarded it to Dr. A. Fain, a noted authority in Belgium. Dr. Fain reaffirmed the material as *A. armillatus* stating that this

Fig. 2.
Immature **Armillifer armillatus** X 1.25 embedded in peritoneum.

appeared to be the first recorded case in a bovine.

It must be stated that a similar infestation was encountered in an ox at the Johannesburg abattoir in 1964, both parietal and visceral serosa of the abdomen being heavily infested [2].

Fortunately infested portions were preserved and mounted so that no difficulty was experienced in identifying this material as an extremely severe infestation of A. *armillatus*.

These cases are recorded for the benefit of our colleagues as it is felt that apart from their interest the parasites may be encountered fairly frequently on the African continent where the definite reptile hosts are common.

ACKNOWLEDGEMENT

Apart from the acknowledgements made in the text, the authors are indebted to the Chief, Veterinary Research Institute, for permission to publish this paper and to Dr. L. W. van den Heever for bringing the case from the Johannesburg abattoir to our notice and making available the preserved material.

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THE OCCURRENCE OF EIMERIA CHINCHILLAE n. sp. (EIMERIIDAE) IN CHINCHILLA LANIGER (MOLINA, 1782) IN SOUTH AFRICA

A. J. DE VOS AND I. B. VAN DER WESTHUIZEN*

SUMMARY

Eimeria chinchillae n. sp. is described from the chinchilla. The oocysts are ovoidal, subspherical or spherical and their mean size is 17.5 by 15.5 microns. The sporocysts are ellipsoidal and average 10 by 6 microns in size. The sporocysts contain a residual body and have a distinct Stieda body.

INTRODUCTION

Eimeria spp. are common parasites of a large number of vertebrates and invertebrates and many species have already been encountered in rodents. Although oocysts have been recorded from chinchillas [3, 4], no detailed account of their morphology has been published. Schofield and Scollard [3] observed oocysts in one animal while investigating giardiasis in chinchillas in the United States of America. Stampa and Hobson [4] found oocysts in chinchillas suffering from digestive disturbances in the Grahamstown district of the Cape Province. They consider the parasite to be pathogenic and discuss the treatment used.

HISTORY

Two dead chinchillas were submitted to this Institute for autopsy on the 20th May, 1967. They originated from a farm in the Pretoria district, where constant mortality was being experienced. Examination of the caecal contents showed a large number of oocysts in both cases. Five days later eight dead chinchillas were submitted from another farm 20 miles from the first. They were also found to harbour oocysts.

In both outbreaks necropsies revealed a marked reddening and thickening of the mucosa of the caecum and initial portion of the colon. In addition, white spots ranging from about 0.5 to 2 mm in diameter were seen in the intestinal mucosa in six of the cases. The caecum and colon were also distended with semisolid ingesta and gas. The rectum contained a small number of faecal pellets coated with mucus.

On both farms the chinchillas were subsequently treated with sulphamezathine in the drinking water at a concentration of 0.24 per cent, as recommended by Stampa and Hobson [4], with apparent success.

MATERIALS AND METHODS

The contents of the caecum and colon were collected from the chinchillas and the oocysts collected by flotation with a saturated saline solution. The oocysts were then washed and incubated at 28° C in Petri dishes containing a thin layer of a 2 per cent aqueous solution of potassium dichromate. Samples were taken at 24 hour intervals and examined for progress in the sporulation of the oocysts.

The illustration (Fig. 1) was made with the aid of a Wild drawing tube.

RESULTS

Description of oocysts: They are ovoidal, subspherical or spherical. The ovoidal forms (Fig. 1) may be flattened at one end and slightly asymmetrical. The wall is light brown in colour, 0.7 to 1.0 micron thick and usually slightly thinner at one or both poles in ovoidal and subspherical forms. No thinning of the wall is detectable in spherical forms. In whole as well as crushed oocysts the wall appears to consist of a single layer only. A micropyle, oocystic residual body and polar granule were not observed. A total of 120 sporulated oocysts measured 13 to 22 microns in length and 11 to 18 microns in width with a mean of 17.5 by 15.5 microns. Their length-width ratios ranged from 1.0 to 1.3, mean 1.13.

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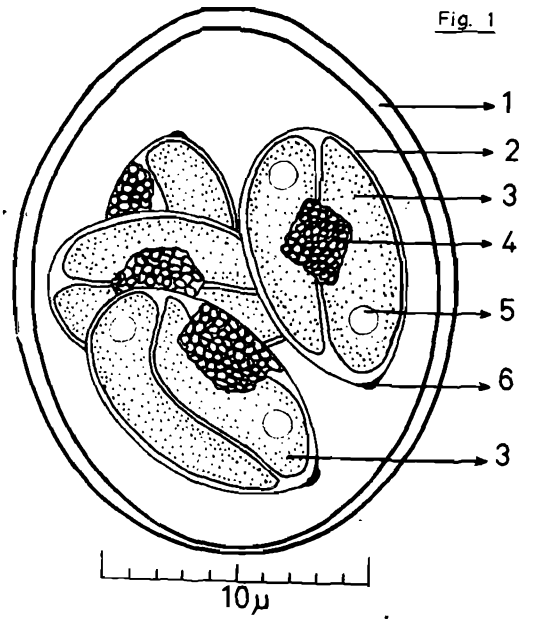
The sporocysts are ellipsoidal to slightly ovoidal with a small but distinct Stieda body at one end. A well defined sporocystic residual body is present and consists of small closely packed granules usually situated near the centre of the sporocyst. Sporozoites are elongated, lie lengthwise in the sporocyst and are sometimes bent double at one end. A small refractile globule is present at one end of most of the sporozoites. Fifty sporocysts measured 8 to 12 by 4 to 8 microns with a mean of 10 by 6 microns. Their length-width ratios ranged from 1.4 to 1.9, mean 1.6.

Fig. 1



Sporulated oocyst of Eimeria chinchillae
1. Oocyst wall 2. Sporocyst
3 Sporozoites 4. Sporocystic residual body
5. Refractile globule 6. Stieda body

Sporulation time: The oocysts are fully sporulated after 72 hours at 28° C. The percentage sporulation of the samples ranged from 65 to 89 per cent.

DISCUSSION

Initially the slight variation in shape and size of the oocysts led us to believe that more than one species might be involved. There is, however, a continuous variation in shape between spherical and ovoidal forms. Furthermore, the frequency distribution curves of the lengths and widths of 120 oocysts (Fig. 2) give no indication that more than a single population is involved. This variation in size is by no means an uncommon feature of a large number of *Eimeria* spp. The morphology of the sporocysts is very similar in spherical and ovoidal oocysts. On the basis of their morphology, we therefore regard these oocysts as belonging to a single species.

Since *Eimeria* spp. are generally regarded as host specific parasites and no species have ever been described in detail from chinchillas, we propose the name *Eimeria chinchillae* n. sp.

This parasite seems to be pathogenic and investigations are being carried out to determine its life cycle and pathogenicity.

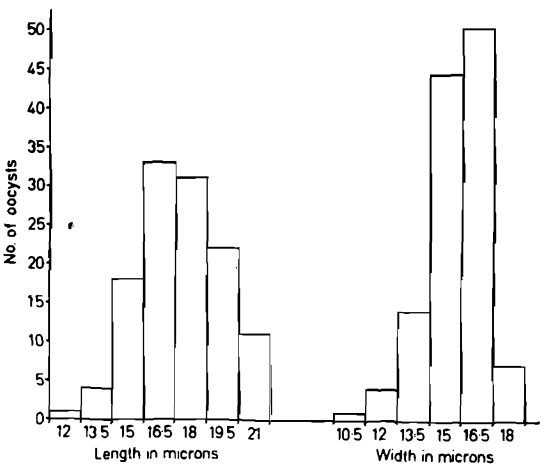


Fig. 2.
Frequency distribution of the lengths and widths of 120 oocysts.

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A NEW BIOLOGICAL METHOD FOR EVALUATING THE EFFICACY OF ACARICIDES AGAINST TICKS

O. G. H. FIEDLER*

SUMMARY

A new technique is described for rearing larval ticks and treating them with acaricidal fluids without having to handle the minute larvae individually. Special envelopes of rice paper, similar to tea bags, are used as containers for the larvae. The method also permits repeated inspections after treatment.

INTRODUCTION

As economically important tick species are displaying resistance to the conventional dipping materials to an ever increasing extent, and, at the same time, new chemical compounds with potential acaricidal properties make their appearance in increasing numbers, research into the susceptibility of the different species of ticks to established and newly evolved acaricides is rapidly gaining importance. Whereas it is easy to handle engorged female ticks and to advise suitable screening methods with them, this task becomes more difficult when unengorged adults and developmental stages are used. The most difficult stage to work with is the minute unengorged larvae which, however, have the advantage that they can be reared in large numbers.

Sufficient numbers of engorged female ticks can as a rule be obtained or reared only with great difficulty and expense, whereas tick larvae would lend themselves to mass screening tests due to their availability if suitable test methods could be found. Such methods should exclude the direct handling of the minute larvae before and after treatment and it should be possible to inspect the test batches at any stage during the process. The larvae of many species of our ticks harbour and transmit African tick-bite fever caused by *Rickettsia conorii* Brumpt, to man. The danger of infection of workers engaged in such screening tests when larvae have to be transferred to new containers at the height of their activity, is evident. Finally, the test should be so designed that it does justice, as far as this is possible, to realities and conditions encountered in practice. Any unrealistic factors to which the larvae are subjected during the test might influence the results to an extent where comparison with conditions encountered in the field is not possible.

It must be stressed that an important criterion for assessing the efficacy of acaricides applied in the field comprises observations as to whether female ticks succeed in reaching a state of engorgement or not. This implies that efforts at control are directed at destroying the immature stages or the unengorged adults before engorgement can take place.

Bearing this in mind, assessment of an acaracide need not necessarily imply mortality of ticks as the only criterion, as paralysis or other profound effect upon the parasies would cause them to drop off the host and thus prevent further parasitism.

After extensive experimentation a technique for treating larval ticks has been evolved which appears to meet the requirements set out above. This paper is released as a basis for further experimentation and discussion only, since the Tick Investigation Centre of the Veterinary Research Institute at Onderstepoort aims at standardizing testing methods employed by bodies concerned[1].

METHOD

As containers for the larval batches under test, thin transparent rice paper **, of the type employed in the manufacture of tea

* Research Department, Agricura Laboratoria Ltd., Box 55, Silverton.

** Dexter and Son, 1 Elm Street, Windsor Locks, Connecticut, 56096, U.S.A.

bags is used. The paper is available as a strip in large rolls with a width of 9.4 cm. To make a bag, the paper strip is folded lengthwise. The open edges on the side are then closed by means of a "paper crimper or welder," either of a rotary or direct pressure type, to form a flat tube, open at both ends. The tube is cut into lengths of 2½ inches which constitute the actual length of the bags, the one open end is then also sealed by means of the crimper. The bag or envelope is now ready to receive the eggs.

Aproximately 100-160 eggs are transferred from the laying tubes into each paper envelope by means of a small scoop. The envelope is then sealed entirely by crimping of the open end. The closed envelopes measure 6.3 x 4.7 cm and the space inside the crimped seams is approximately 5 x 3.8 cm. A piece of stiff white paper or card measuring about 4.7 x 1.9 cm is stapled to one of the short sides which now forms the upper end, whereas the cluster of eggs is situated near the lower end. Through a hole pierced in the middle of the upper edge of the paper strip, the narrow end of a paper clip is inserted to act as a hanger. All particulars regarding the egg batch are written on the paper strip. A completed envelope is shown in fig. 1. The labelled bags are hung by means of the paper clips onto rods suspended within an aquarium (fig. 3). Aquaria measuring 24 x 12 x 12 inches are suitable for this purpose and 18-20 bags can be hung per rod, enabling a tank of this size to take at least 20 rods and thus approximately 400 bags.

The tanks are stored at a constant temperature of 26° C and a relative humidity of 60%. About ¼ inch of water covers the bottoms of the tanks which are partially sealed above by a layer of cotton wool beneath a glass lid to prevent evaporation and to ensure a relative humidity within of practically saturation point. (92-94%).

Hatching and movement of the larvae can be observed through the glass sides of the aquarium with the naked eye. During the third week after hatching the larvae are ready for the tests[2]. By this time the larvae will have separated from the egg shells and will be moving actively in the upper part of their envelopes, (fig. 2). If they are not crawling around in the expected manner, they can be activated within minutes by exposing the envelopes to sunlight.

The larvae are now treaaed by dipping the envelopes into the dipwash to be evaluated. As the mass of empty egg shells is inclined to cause the formation of an air bubble inside the envelope during the process of dipping, thus preventing some larvae from coming into intimate contact with the dipping fluid, the lower ½ inch of the bag containing the egg shells is separated from the top by crimping a second seam about ½ inch above the bottom seam as indicated by the black line in fig. 2. A crawling space for the larvae of 3.8 x 3.8 cm thus remains inside the envelope. Any dead larvae collect at the bottom and will thus be excluded from the test at the same time. The separated lower portion can be left or simply cut off.

During immersion in the dipping fluid or in plain water, penetration through the rice paper is immediate as the bag is being submerged. The rice paper absorbs and retains an average of 0.01 ml per cm^2. The dipwash has no influence on the paper envelopes, which neither warp nor open during the dipping process. Each bag is immersed once only for a couple of seconds, and hung up immediately on a rod in a dry aquarium (26° C and 60% RH). Within approximately 70 minutes the envelopes are dry and are transferred to a humidified tank and kept for subsequent observation. The envelopes can be removed from the storage tank at any time for examination under a stereomicroscope and put back again for further observations. The small larvae as well as details of their movement can be seen through the dry rice paper in transmited light at a magnification of 6 to 16 times. To facilitate counting, the envelope is placed on a sheet of translucent material (Perspex) which is fitted with black recticles about ¼ inch apart and which can be moved freely on the stage of the microscope

The normal handling of the paper envelopes from the storage tank to the microscope is usually sufficient stimulation to induce movement of those larvae which are still alive. The bag may also be shaken slightly to induce movement. The dead and severely affected larvae always fall downward and lie at the lower end of the envelope while the unaffected specimens normally crawl around in the upper part.

DISCUSSION

The larval immersion technique presented here differs in several respects from the methods applied until now[1-4]. The larvae

are neither handled to transfer them from the breeding tubes to filterpaper sandwiches for dipping, nor individually removed from the treated paper to new paper discs folded into segments for storage. Time and labour are saved in this way and the hazard of escaped ticks transmitting tick-bite fever is greatly reduced.

The method makes provision for the treatment of living, active larvae only, and all the specimens can be inspected later at any time and as often as required. The mode of action of the acaricide can be observed on a single batch, thus excluding the large number of repetitions necessary when each new batch can be inspected once only. Acaricides with a rapid or slow action can therefore be more easily determined. With the method described, mortality depends upon the acaricidal residues on the body of the larvae and on the fibres of the rice paper; this closely simulates conditions encountered on the body of the animal. Malan [1] has shown that these residues are in fact mainly responsible for the control of the larvae and that the time of immersion plays no rôle. For this reason, and due to rice paper being readily permeable to any aqueous liquid, the time of immersion is reduced to a matter of seconds only.

The temporary "knock-down" effect following dipping described by Malan [1] plays no part with the new technique, because no selected specimens are removed for observation, as practised by Shaw [2]. The removal of a number of larvae as they begin to crawl after temporary immobilisation could be responsible for a substantial experimental error, as the tendency is to select for evaluation only the less susceptible and more robust specimens, which are the first to recover.

Formation of moulds was not experienced in the rice paper envelopes at near saturation humidities, as a certain degree of circulation and exchange of air is possible through the porous walls.

With conventional techniques the assessment of mortality is made by cutting open the sealed filter paper and counting dead and living larvae. Shaw [2] classified those showing movement as alive, those failing to move as dead. Unfortunately he does not explain exactly what he means by the term "movement". Malan [1], on the other hand, maintains that a larva not showing movement is not necessarily dead, as it may only be affected to some degree by the acaricide and does not move voluntarily. Some of these larvae, however, will show movement when stimulated by the touch of a needle. His final criterion for death, therefore, is no movement after stimulation.

Experience has taught that it is rather difficult and not very reliable to classify the various degrees of injury produced by an insecticidal compound. The decision will always be subjective and differ according to the observer. Furthermore, the techniques used to date tend to penalize slow acting compounds which otherwise may be potent remedies in the field.

A better criterion of the extent of injury is to determine the number of specimens which have survived the treatment. All specimens that display a natural posture are classified as "alive", whereas all the others (affected, knocked-down, moribund and dead) are classified as "dead". The period that has to elapse before such a reading can be taken, is in excess of the 18 hours so far applied and has to be determined to suit each species. The technique described lends itself to this type of assessment, as all affected and dead specimens will collect at the bottom of the bag and all larvae that are still viable will frequent the upper part of the bag, following their natural trend. Viable larvae that are inactive can be activated by exposing them to a higher temperature and light.

The paper envelopes are suitable for larger mites and small insects as well.

ACKNOWLEDGEMENTS

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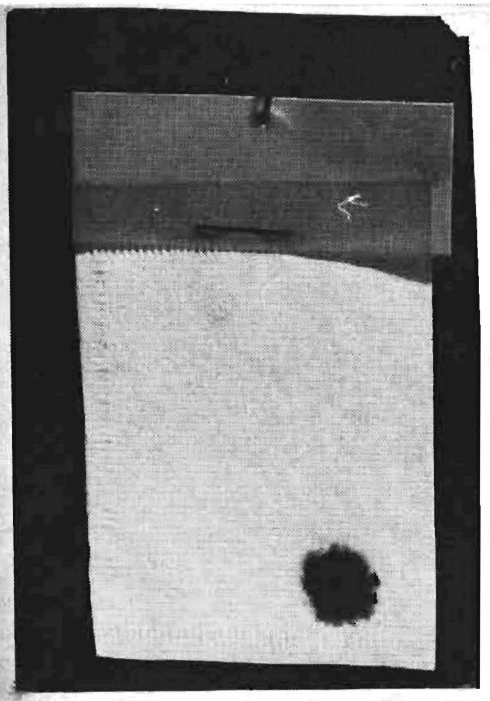

Fig. 1

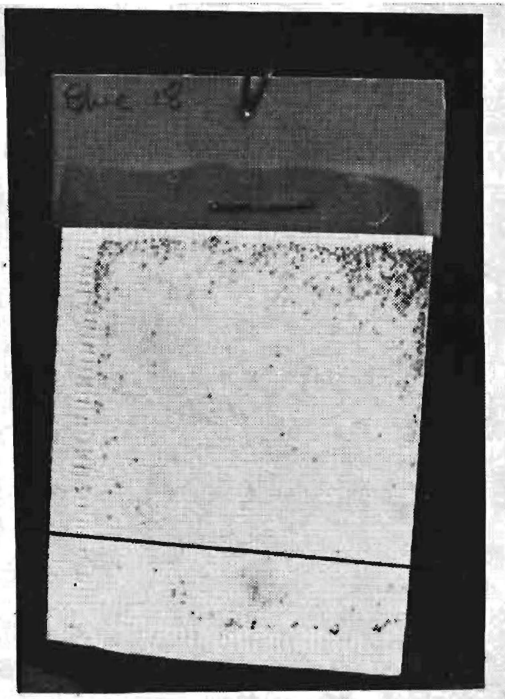

Fig. 2

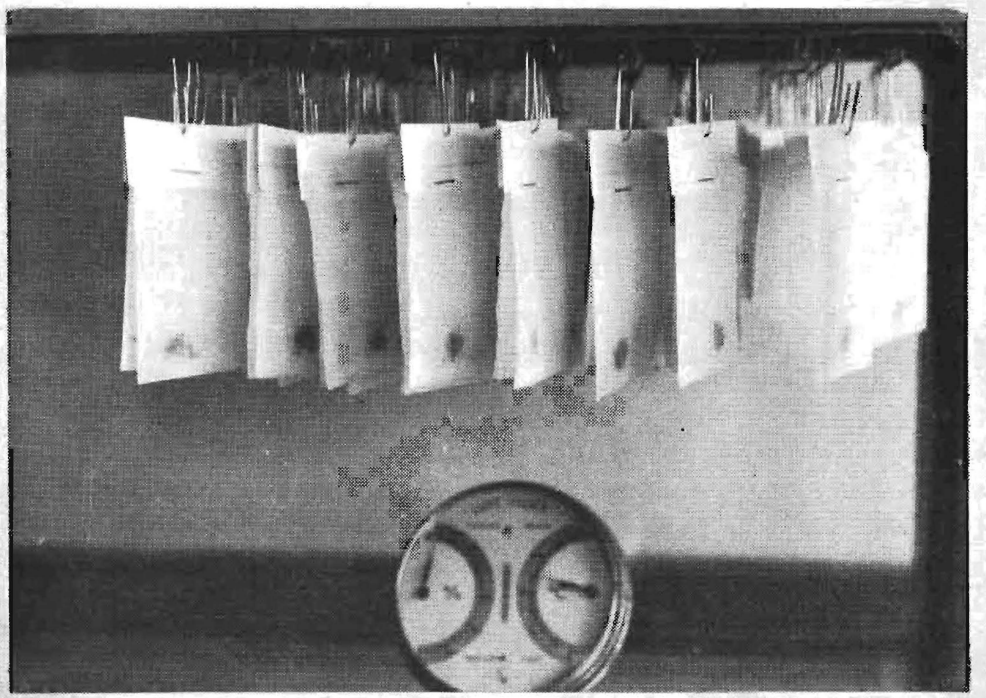

Fig. 3

LEGEND

The black line indicates the position of the second seam ½ inch above the lower end of the bag.

Fig. 1. — Rice paper bag with a batch of tick eggs.

Fig. 2. — Rice paper bag with living larval ticks.

Fig. 3. — Storage of rice paper bags prior to hatching.

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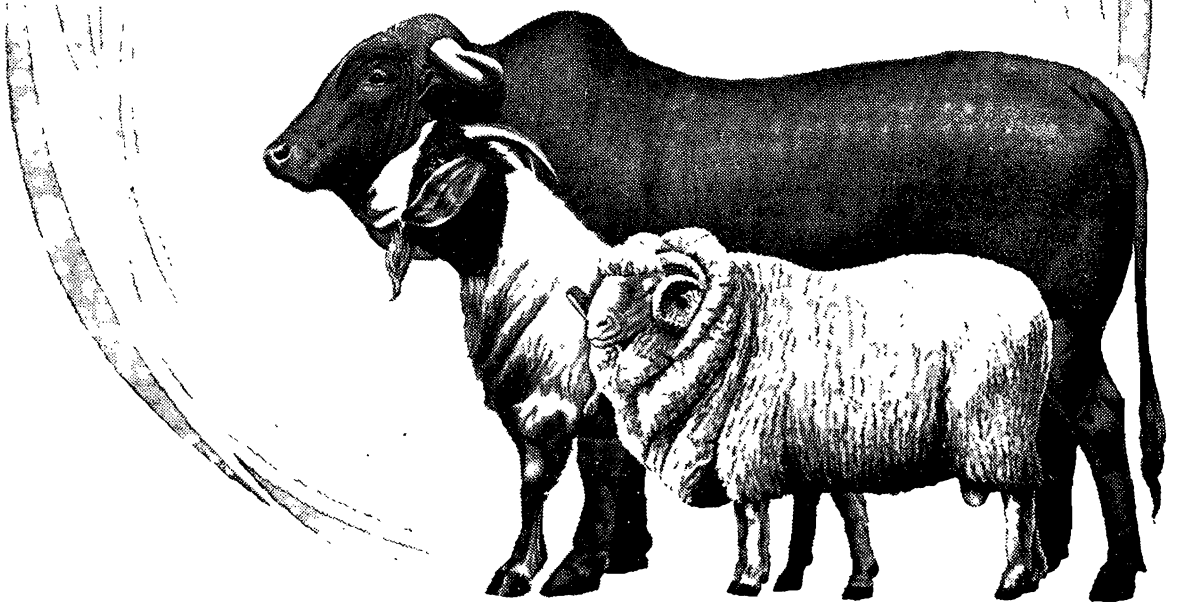

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A NOTE ON AN ABNORMAL HAEMOGLOBIN IN CASES OF THE GEELDIKKOP—ENZOOTIC ICTERUS DISEASE COMPLEX

L. P. NEETHLING*, J. M. M. BROWN**, D. R. OSTERHOFF**, P. J. DE WET** AND I. S. WARD-COX**

During the course of our investigations into the disturbed biochemistry of geeldikkop and enzootic icterus in sheep we studied the haemoglobin types in 24 substantially worm-free Merino sheep which had recovered from an attack of geeldikkop. These animals were studied by us four months after the acute manifestations of the disease. Haemoglobin phenotypes were studied by means of the standard starch gel electrophoresis technique and using the Buschmann [1] continuous buffer, staining with amido black 10 B and reading on the bottom 1.5 slice.

We found three AA, eight BB and thirteen AB phenotypes of which four were of the type ABC and two were of the type AAC. We have designated this abnormal haemoglobin band the C band in accordance with the nomenclature of Huisman [2], which is identical to the haemoglobin N band of Braend, Efremov and Helle [3].

Since the existence of a haemolytic syndrome in geeldikkop was postulated earlier [4], these findings were not totally unexpected. Hitherto this abnormal haemoglobin has only been found in sheep following massive exsanguination or in sheep with heavy intestinal parasite infestation, primarily with *Haemonchus contortus* [2].

The abnormal haemoglobin phenotype appeared to constitute between 5-80 per cent of the total haemoglobin present in our affected animals. The C-haemoglobin disappeared completely from the blood of these animals during the course of the following three months, the rate of disappearance being directly related to the concentration of C-haemoglobin in the blood.

The presence of the abnormal haemoglobin in these cases four months after the acute phase of the syndrome is evidence of the severity of the haemolytic process previously postulated [3] as well as its continued existence in these animals for an undefined period.

To the best of our knowledge this is the first report of the occurence of this C-haemoglobin in a specific disease of sheep associated with various biochemical lesions. Detailed studies on the association of the abnormal haemoglobin type with geeldikkop and enzootic icterus and its experimental induction will form the subject of a later report.

ACKNOWLEDGEMENTS

The Chief, Veterinary Research Institute, Onderstepoort, is thanked for permission to publish this note.

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THIODAN* POISONING OF CATTLE — A CASE REPORT

J. TERBLANCHE AND J. A. MINNE**

SUMMARY

Three bovines died within 15 hours after ingesting approximately 15.9 kg of peanut hay containing 160 mg of Thiodan per 100 g of hay. A sheep to which Thiodan was administered at the rate of 8 mg/kg for two days showed marked symptoms of toxicity including convulsions.

The report has special reference to the toxic dose of Thiodan* (Endosulfan) for ruminants by the oral route.

INTRODUCTION

The toxicity of Thiodan for bovines has only been documented twice [1, 2] since its technical description in 1957. A third case is recorded in the Toxicology section at Onderstepoort. Two of the above mentioned cases were due to the accidental spraying of cattle with Thiodan instead of a benzene hexachloride miscible oil [1, 4]. A fourth incident of Thiodan poisoning is presented in this paper; details of toxicity trials on sheep are also provided.

HISTORY AND SYMPTOMS

At 5 p.m. on Sunday evening the three cows appeared normal. At 7 a.m. the next morning one cow was found dead, while the second was in a state of prostration. At 8.45 a.m. the third animal had a sudden convulsive and fell, causing injury over the bony prominences of the body. The convulsions, which soon subsided, were clonic in nature, leaving the animal inco-ordinated. During the convulsions there were violent blepharospasms and fasiculations of the facial muscles. The same nervous symptoms have been observed in cases of peracute lead poisoning [5].

Yellow maize meal and Rumevite † had been mixed on the farm. Suspecting this mixed concentrate to contain a large quantity of urea, a tentative diagnosis of urea poisoning was made. Treatment with 10 per cent acetic acid was of no avail and both animals died.

Late on Sunday afternoon the animals had received 15.9 kg of groundnut hay which had not been fed before. The cow found dead the next morning was the first animal to have eaten from the hay. The three autopsies performed provided no indication to the cause of the sudden death.

CHEMICAL AND BIOLOGICAL TESTS

The following specimens were collected: the remainder of the groundnut hay, concentrate mixture, and liver and kidney post *mortem*.

A. *Biological Tests*

Two Merino sheep were dosed by stomach tube with finely ground quantities of the suspect peanut hay.

1. A 3 year old wether received 180 g of the peanut hay at 5 g/kg for two days. The first signs of toxicity were observed 24 hours after dosage: severe depression, blindness, and anorexia, with moderate clonic convulsions following soon afterwards. Aimless wandering became apparent when the animal was let out into a paddock. After one more seizure on the same day and after appearing apathetic for a further two days, the sheep made an uneventful recovery.

2. A second wether, aged 2 years received 290 g of the peanut hay at 10 g/kg. No signs of ill health were observed during

* Thiodan: (Endosulfan) hexachloro, hexahydro-methanobenzo-dioxathiepinoxide. (A plant protection insecticide).

** Veterinary Research Institute.

† Rumevite (National Products, Germiston) a supplement containing urea.

†† Sagatal: (Sodium pentobarbitone) May and Baker.

‡ Calciumborogluconate: May and Baker.

the following two days. The same dosage was then repeated on the third day. Eighteen hours later the animal was found in a state of hyperexcitability, with twitching of the ears and excessive salivation. Violent clonic convulsions occurred soon afterwards resulting in severe anoxia; 5 ml Sagatal†† administered intravenously controlled the convulsions whereafter the respiratory embarrassment was arrested. After aditional administration of calciumboro-gluconate†* the animal recovered. The convulsions were violent enough to have killed the animal. Seizures occurring later in the day were suppressed by Sagatal ††.

B. *Chemical Tests:* Those undertaken are summarised as follows:

1. No urea could be detected in the concentrate.

2. The organs analysed proved to be negative for lead.

3. On sublimation, the peanut hay yielded a crystalline substance with a low melting point (85°) containing sulphur and chlorine.

4. An infra-red spectrum of the crystalline material obtained on a Hilger and Watts photometer was found to be identical to that of technical Thiodan.

5. Using the colorimetric method of Maitlen and Walker[6], the toxic peanut hay was found to contain 160 mg of Thiodan per 100 g of peanut hay.

DISCUSSION

This is the first recorded case of fatal Thiodan poisoning to occur in South Africa after the ingestion of toxic material. The symptoms observed were not unlike those one would expect to see with chlorinated hydrocarbon poisoning.

Signs of toxicity were observed in a sheep receiving 5 mg/kg for two days. A second sheep which received a single dose of 10 mg/kg appeared normal for two days. The animal acted most violently when the dosage was repeated on the third day, and would have succumed had the convulsions not been controlled with Sagatal.

The three cows consumed approximately 15.9 kg of the toxic hay containing 160 mg of Thiodan per 100 g of hay. The weight of the cattle were estimated at 275.6 kg, 318 kg and 275 kg respectively: each cow had thus ingested 29.5 mg/kg of Thiodan which proved to be lethal. This figure is much lower than the $L.D._{50}$ for white rats which is reported to be 40-50 mg/kg[3]. A steer ingesting 5 mg per kg of Thiodan for 2 days was observed to show inco-ordination, muscle tremor, and excessive salivation[2].

Taking into account that a sheep dosed twice, at the rate of 10 mg/kg over a period of 4 days, developed convulsions severe enough to kill the animal, it may well be assumed that a single dose of 30 mg of Thiodan/kg would be fatal to a bovine.

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NATURAL OCCURRENCE OF SELENIUM IN SHEEP BLOOD AND TISSUES AND ITS POSSIBLE BIOLOGICAL EFFECTS

L. P. NEETHLING*, J. M. M. BROWN** AND P. J. DE WET**

SUMMARY

Selenium levels in the tissues of sheep emanating from various parts of South Africa were studied by neutron activation analysis. The distribution of the element in sheep tissues is indicated. Some thoughts are expressed as to the nature of selenium compounds present in the natural pastures and their possible role in the pathogenesis of geeldikkop and enzootic icterus.

INTRODUCTION

The use of radio-activation analysis has made it possible to determine selenium with a high degree of accuracy in various biological materials. We have developed a procedure of this nature whereby the analysis of selenium in blood and tissues of sheep can be done non-destructively. This is a report on the occurrence of selenium in sheep from areas where geeldikkop and enzootic icterus are prevalent and in animals raised or maintained elsewhere.

MATERIALS AND METHODS

Four farms were selected for study in parts of the Karoo where the ovine diseases, enzootic icterus and geeldikkop, occur regularly. Two of these farms, numbers 1 and 2, were located in the Hofmeyer district. Losses due to either syndrome on these farms were minimal during the season 1966-67. The other two farms, numbers 3 and 4, were situated near Murraysburg. Stock losses due to the two diseases mentioned were heavy during the last two seasons. A fifth farm, number 5, was selected as a control farm. Losses due to the disease complex were minimal over a period of a number of years. The sheep studied on all of these farms were 15-34 month old Merino ewes which were grazed on Karoo veld typical of the areas concerned [1]. Very few of these animals had experienced a clinically detectable attack of either disease. Heparinized blood samples were obtained from these animals during the first week of December in 1967 for selenium analysis. At this time no cases of either syndrome were reported in the areas concerned.

A group of 25 adult Merino ewes and wethers were drawn from the pool of available animals at Onderstepoort to serve as controls. These animals had been maintained at Onderstepoort for a number of years on a diet of lucerne hay, teff hay and water *ad libitum*.

In addition , two sheep were obtained for organ analysis from farms at Murraysburg and Kroonstad and a further one was drawn from the Onderstepoort pool for the same purpose.

All blood samples were centrifuged, the cells removed and washed once with saline which was removed as completely as possible at the expense of some of the red cell precipitate after centrifugation. Approximately 3 ml of packed cells from each sample were freeze-dried in the conventional way. Accurately weighed amounts of the dried red cells in the order of 400 mg were transferred to polythene capsules measuring 2.2 cm long x 1.0 cm internal diameter and closed with a tight fitting screw cap. A limited number of plasma samples were treated in the same manner.

As selenium standard a solution of selenium dioxide (minimum assay 99% ex Se) containing 1 ppm Se was prepared by accurate dilution. Varying amounts of from 0.2 to 2.0 ppm Se were sealed into polythene capsules for use in obtaining the calibration curve.

Tissue samples in the order of 1.0 g were taken and placed into the screw capped polythene containers mentioned above immediately after slaughter of the animals concerned.

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Samples were irradiated in a flux of approximately 10^{13} neutrons/cm^2/sec using the 5 MW "Safari 1" (ORR) reactor. The rapid-transit pneumatic "rabbit" system used had a Wescott epithermal index (r) equal to 0.0148. Each sample was irradiated for 20 seconds followed by a 25 second cooling time, after which a 30 second live time count began. A 3 x 3 NaI (Tl) scintillation crystal and one fourth of the channels of a RIDL 400 channel γ-ray spectrometer served as counting equipment. The γ-energy spectrum was observed from 0-300 KeV. The number of counts in the Se-77m photopeak at approximately 0.17 MeV were estimated by summing the top 10 channel counts and substracting as background 10 times half the number of counts in the adjacent first channel on both sides of the photopeak.

After we had satisfied ourselves that the method gave reproducible results by repeated irradiation of a set of standards, the various samples were analysed. A standard was irradiated after every 10 samples as a further precaution and to ensure that no changes in the neutron flux had occurred.

Glyceraldehyde-3-phosphate dehydrogenase was isolated from the gluteal muscles of two sheep by the method of Racker [2]. One of the animals was drawn from the Onderstepoort pool, the other was a case of enzootic icterus emanating from Carnarvon. The dried enzyme preparation was treated in the same manner as the red blood cells.

Interpretation of the statistical evaluation of the experimental data was performed as described previously [3].

RESULTS

These are summarised in Tables 1, 2 and 3.

DISCUSSION

This is the first time that selenium has been determined on the erythrocytes of sheep using selenium-77m as indicator in non-destructive activation analysis. A search through the literature brought to light similar work on human red cells and tissues [4]. Levels of selenium in the erythrocytes of other mammals do not appear to have been determined by this method.

The values found for the erythrocytes of our control animals shown in Table 1 represent 0.25 mcg Se/g intact red cells for Onderstepoort sheep and 0.27 mcg Se/g intact red cells for sheep from the Control Farm number 5. These values compare favourably with the value of 0.23 mcg Se/g intact red cells reported as the mean selenium content of the erythrocytes of 254 normal humans [4, 5]. Since the figures from our control animals were obtained from clinically healthy sheep maintained under good conditions of nutrition and management, we accept for the purposes of this discussion that

Table 1.—SELENIUM LEVELS IN THE ERYTHROCYTES OF SHEEP FROM DIFFERENT LOCALITIES.

(Values for Selenium are expressed as mcg Se/g Dried Erythrocytes. To obtain mcg Se/g Intact Red Cells divide by 3.2)

LOCALITIES	Number of samples	Mean Selenium level	Standard deviation	Statistical significance when compared with control localities
Farm No. 1 (Hofmeyr)	50	1.68	0.37	Highly significant
Farm No. 2 (Hofmeyr)	64	1.29	0.38	Highly significant
Farm No. 3 (Murraysburg)	33	1.10	0.26	Significant
Farm No. 4 (Murraysburg)	67	1.26	0.28	Highly significant
Farm No. 5 (Middelburg)	12	0.85	0.37	
Onderstepoort	25	0.81	0.30	

Table 2.—SELENIUM LEVELS IN THE ERYTHROCYTES OF SHEEP OF DIFFERENT AGES.
(Values are mcg/g Dried Red Cells. To obtain mcg Se/g Intact Erythrocytes, divide by 3.2).

AGE GROUP Approximate age	Mean Selenium Value	Standard Deviation	Number of Samples
15—16 months	1.33	0.31	41
21—22 months	1.40	0.65	73
27—28 months	1.37	0.47	35
33 months and older	1.28	0.43	47

Table 3.—THE DISTRIBUTION OF SELENIUM IN THE TISSUES OF SHEEP TAKEN FROM THREE DIFFERENT LOCALITIES.

(Values are mcg/g of Wet Tissue).

Tissue	Onderstepoort Sheep	Kroonstad Sheep	Murraysburg Sheep
Kidney	1.75	1.48	2.45
Thyroid	1.35	0.93	1.26
Brain	0.05	0.56	0.75
Liver	0.91	0.53	2.03
Aorta	1.80	0.51	4.33
Pancreas	1.76	0.49	2.56
Ovary	0.47	0.46	0.50
Spleen	1.17	0.40	1.09
Adrenal	0.57	0.18	0.98
Gluteal Muscle	0.47	0.18	1.04
Total Selenium in the above tissues	10.30	5.72	16.99
Ratios of Total Selenium	1.8	1.0	3.0

the normal range for selenium in sheep erythrocytes under such conditions is 0.26 ± 0.10 mcg Se/g intact red cells.

The erythrocytes of the animals from farms 1 to 4 gave mean selenium levels ranging from 0.39-0.53 mcg Se/g intact red cells. This is approximately a factor of 1.8 times the normal level. Whether selenium is regarded as an element with definite biological functions or not, the elevation of its levels in erythrocytes from the control values to nearly double could be expected to influence biological functions in the body.

It has been postulated that there is good agreement between the occurrence of geeldikkop and enzootic icterus and the levels of selenium in sheep tissues [1, 6]. The figures given in Tables 1-3 place this contention beyond doubt. Although the values we report in this paper are not absolute values, they can be considered closer to the true values for selenium in the tissues examined than the earlier published fgures, [1, 6, 7] which, although also relative, are too high.

Table 2 depicts an analysis of the distribution of selenium in red cells of sheep within various age groups. Although the differences in this respect between the various groups are not significant, one can see definite peak levels of the element in the red cells of the age group 21-22 months. In the older animals erythrocyte selenium levels appear to decline somewhat. This may be associated with a cumulative selenium intoxication and the death of a considerable section of the affected population once a certain threshold value has been reached. The fact that no statistically significant differences between the various age groups exists is in agreement with the data published by Dickson and Tomlinson who found no differences in selenium levels in the red cells of male and female humans of different ages.

Selenium was determined on a small number of plasma samples from the various animals studied. On the whole, plasma selenium levels are lower than those of the erythrocytes. Typical values of 0.50 mcg Se/g freeze-dried plasma were obtained for Onderstepoort sheep as against values of 0.78 and 0.85 mcg Se/g freeze-dried plasma for cases of geeldikkop and enzootic icterus.

Table 3 contains a comparison of selenium levels in the various organs of three animals. The one designated "Onderstepoort" originally came from the Laingsburg area some three years before slaughter. This area adjoins the regions where geeldikkop is enzootic. The "Kroonstad" sheep emanated from an area in which geeldikkop and enzootic icterus have, to our knowledge, never occurred naturally and one in which no evidence has been brought forward to suggest the existence of selenium deficiencies or excesses in the natural pastures. The "Murraysburg" animal came from a farm on which geeldikkop is naturally rife, and was slaughtered directly after arrival at Onderstepoort (as was the Kroonstad sheep.)

For the purpose of this discussion we assume that the values given for the tissues of the Kroonstad sheep are "normal" with respect to animals on a nutritionally adequate and non-toxic selenium intake. Assuming the ten tissues listed in Table 3 to make up a representative sample of the whole body, it is clear that the selenium status of these three sheep differ widely. Taking the total selenium content of the Kroonstad samples as 1.0 the respective values for the Onderstepoort and Murraysburg samples become 1.8 and 3.0 This clearly indicates the marked elevation of selenium levels in the body tissues of animals emanating from areas where geeldikkop and enzootic icterus are prevalent.

The data in this table are in general accord with previously published distributions of selenium in tissues like kidney, liver, pancreas, gonads and thyroid [5, 8]. The markedly increased values found for the various tissues of the Murraysburg sheep are thus to be expected under conditions of increased selenium intake [1, 7]. The high concentration of the element in aortic tissue is however remarkable, particularly in view of the fact that this tissue consists largely of collagen, an extreme example of metabolic stability [9]. In view of the recent work by Hansson and Jacobsson [10, 14] on the distribution of administered organic and inorganic selenium in organs of rats we feel that the distribution of selenium in sheep tissues as shown is consistent with the intake of organic selenium. We must therefore conclude that the selenium present in the natural pastures concerned is mainly organic in nature.

It has been postulated earlier [6] that a biochemical feature of geeldikkop and enzootic icterus is an inactivation of certain sulfhydryl-group containing dehydrogenases by selenium. Glyceraldehyde-3-phosphate dehydrogenase was isolated simultaneously from gluteal muscle tissue of a sheep drawn from the Onderstepoort pool and a case of enzootic

icterus. The selenium content of the enzyme preparations was found to be 0.32 mcg Se/g dry matter and 0.76 mcg Se/g dry matter respectively. This increase of selenium by a factor of 2.4 in the enzootic icterus case can probably alter the activity of this enzyme. This could result from displacement of sulphur in active -SH groups or from secondary structural changes in the molecule as has been shown to occur in the case of seleno-β-galactosidase by Huber and Criddle [11].

From all the evidence available it is clear that the administration of organic selenium to animals results mainly in its incorporation in body proteins [12, 13, 14]. Although the chemistry of selenium and sulphur is similar in many respects there are quite a few marked differences with regard to physical characteristics like, for instance, covalent radius, electro-negativity and hydrogen bonding ability [15, 16]. The replacement of sulphur by selenium can thus induce alterations in certain fundamental protein functions which we know are highly dependant, for instance, on phenomena like intra- and intermolecular hydrogen bonding or inter-molecular distances and steric configurations. The specific function of antibodies depends upon these particular structural considerations. The two long and two short chains in antibodies are linked together by at least four disulphide bridges representing a minimum of eight sulphur atoms [17]. Replacement of one or more of these atoms by selenium atoms must necessarily result in a structural change in the macromolecule. Depending upon the number of replaced atoms the specificity of the antibody must be altered.

The presece of an abnormally large pool of selenium in an animal could result in the production of abnormal antibodies, having lost their original specificity. On the other hand the abnormal proteins in such a body could result in antigen formation giving rise to foreign antibodies. Both of these factors appear to be operative in the pathogenesis of the haemolytic episodes in geeldikkop and enzootic icterus possibly in setting up an auto-immune state such as has been demonstrated in a number of recurrent haemolytic conditions in humans [18, 19]. The existence of an auto-immune haemolytic syndrome in Karoo sheep can be reconciled with all that is known of the epizoology, symptomatology and biochemistry of geeldikkop and enzootic icterus. Preliminary work initiated by these ideas supports this hypothesis.

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THE ENIGMA OF OESTRUS MACDONALDI GEDOELST SOLVED

F. ZUMPT*

In 1912, Gedoelst [1] based his description of a new oestrid species which he named *Oestrus macdonaldi,* on the third larval stage. Several specimens had been found in the nasal cavities of Lichtenstein's hartebeest, *Alcelaphus lichtensteinii* (Peters), at Lupula, former Belgium Congo. As a characteristic feature, Gedoelst revealed the presence of a double row of spinules on the dorsal side of segment II, and the absence of rows or patches of spines on the dorsal sides of segments III to IV or V, which distinguishes it readily from the third larval instars of *Oestrus aureoagentatus* Rodhain & Bequaert and *O. caucasicus* Grunin. In my book on "Myiasis in man and animals of the Old World", I have drawn up a key for the 3rd larval instars of *Oestrus,* and *O. macdonadi* found its place between *O. variolosus* (Loew) and *O. ovis* Linnaeus. These three larval forms are morphologically very similar to one another, and seem moreover to be subject to a certain intraspecific variability, which in some specimens causes an overlapping of the taxonomic features. In the 3rd larval stage of *O. macdonaldi,* the above mentioned dorsal spinules on segment II apparently forms a constant feature but, according to my material, sometimes only one row is clearly developed. In *Oestrus ovis* this feature is only poorly developed or those spinules are not present at all. Specimens of *Oestrus ovis* with a few dorsal spinules are, however, separable from *O. macdonaldi* by only three to four ventral rows of spines on segments VI to VIII, whereas there are five to six or even seven in *O. macdonaldi.* A careful examination is also necessary in respect to the 3rd larval instars of *O. ovis* and *O. variolosus,* where the second segment is completely bare dorsally in *O. ovis.* In these speciments, the number of spines on the median post-anal bulge gives an additional character for separation.

The hatching of the adult flies, which should always be tried, makes the identification an easy matter. In this stage *O. variolosus* is always readily separable from *O. ovis,* and so far no *O. ovis* have been reared from wild antelopes, conversely no *O. variolosus* from sheep. Records in the literature of African antelopes forming a reservoir for *O. ovis,* have evidently been based on faulty identifications from the 3rd larval instars, on the other hand, I have never received oestrid larvae from sheep and goat in subsaharan Africa which could be referred to *Oestrus* species known to occur in antelopes.

In my key to the 3rd larval instars, *Oestrus bassoni* Zumpt had been omitted. This species was based on two males and two females, which show very characteristic features and are easily recognizable [3]. The females had been detected in the collection of the Zoological Museum of Berlin and were found in the Cape in the beginning of the last century; the two males had been reared from larvae recovered from a Red hartebeest, *Alcelaphus buselaphus* (Pallas) at Mariental, S.W. Africa, by Dr. P. A. Basson, Onderstepoort.

Unfortunately, no larvae had been preserved of the series from which the adults of *O. bassoni* had been hatched. Dr. Basson, however, had sent me some which he thought to belong to *O. bassoni,* but they were *O. macdonaldi.*

In July 1967, the Director of the Nature Conservation Branch, Transvaal Provincial Administration, informed me that on a private farm near Krugersdorp several blesbok, *Damaliscus dorcas* (Pallas) had to be shot and kindly offered to let me examine these animals for parasites. One of my assistants, Mr. J. Ledger, attended the procedure and isolated a number of larvae from the nasal cavities on sand for hatching and in alcohol for studying the larval features. Fortunately,

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five males and two females of *O. bassoni* hatched, and one female of the common *Gedoelstia haessleri* Gedoelst. The identification of the larvae also resulted in two clearly separable species, namely *O. macdonaldi* and *G. haessleri.* From these findings it is now absolutely clear that *O. macdonaldi* represents the larval form of *O. bassoni.* According to the rules of nomenclature, *O. macdonaldi* is the valid name, and *O. bassoni* its synonym.

Oestrus macdonaldi is so far known from the following hosts and localities[2].

Alcelaphus lichtensteinii (Peters) — Congo,

Aldelaphus buselaphus (Pallas) — S.W. Africa,

Damaliscus dorcas (Pallas) — Transvaal,

Damaliscus korrigum (Ogilby) — Tanzania.

Oestrus macdonaldi is an uncommon species and probably only scantily distributed. It is certainly worthwhile to look for it and to recover more material by preserving the larvae in 70% alcohol and rearing adults from the relevant larval series.

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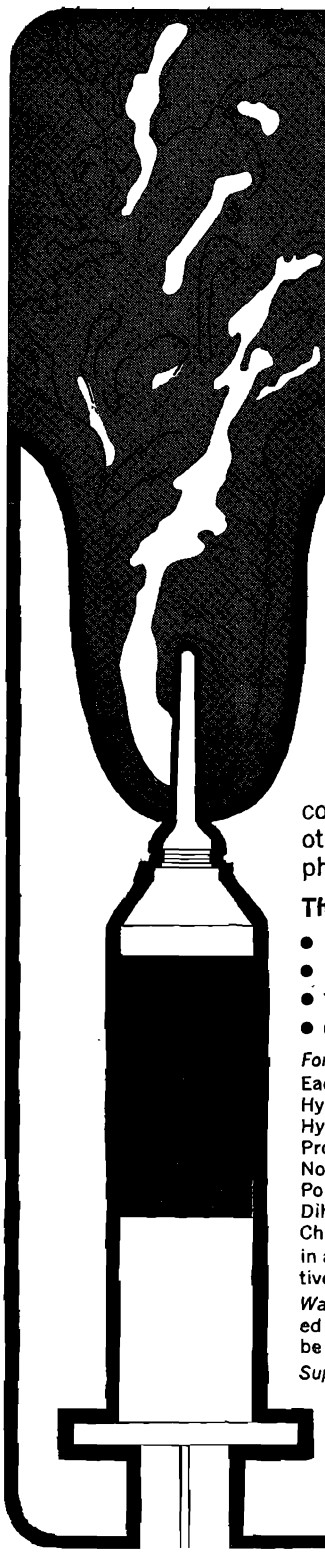

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AN OUTBREAK OF CLAVICEPS PASPALI POISONING (PASPALUM STAGGERS) IN BEEF CATTLE

W. J. EHRET*, T. F. ADELAAR** AND N. P. J. KRIEK**

SUMMARY

An outbreak of 39 cases of *Claviceps paspali* poisoning amongst beef cattle on a large semi-intensive irrigation farm is described. Predisposing conditions, symptomatology, control measures, and the experimental reproduction of the disease in two bovines are discussed.

INTRODUCTION

Claviceps paspali (ergot) is a fungus which parasitises the seed heads of *Paspalum* grass species. The degree of infection varies widely with climatic conditions, being heaviest after wet humid summers [1, 2].

According to Blood and Henderson [3] the ergots are most toxic when they are passing from the sticky 'honey-dew' (sphacelial) stage to the hard brownish sclerotial stage (2-4 mm in diameter).

Cattle are the species most commonly affected in the field. An outbreak of poisoning in South Africa due to *Claviceps* species on *Paspalum* grass was reported in 1956. Experimental reproduction of the disease in livestock has been recorded previously [5, 6]. Horses and donkeys proved to be the most susceptible, followed by cattle and pigs in decreasing order [5]. Blood and Henderson [3] state that sheep are also susceptible.

The clinical signs are manifestations of nervous derangement, consisting of initial transient depression which changes rapidly to increased reflex stimulation of the central nervous system, disturbance in co-ordination, convulsions and, in severe cases, general paralysis terminating in death [5].

HISTORY

The outbreak occurred in a herd of 783 maiden heifers on a cattle breeding and fattening scheme which the City Council of Johannesburg operates in conjunction with sewage and waste water purification.

The management system includes the rotational strip grazing of irrigated exotic pastures. The pasture where the outbreak occurred consists mainly of perennial rye grass *(Lolium perenne)*, patches of fescue grass and large areas which, due to the moist conditions, have become infiltrated by *Paspalum dilatatum* (dallas grass) and *P. distichum* (water-couch grass). An estimated 45 inches of effluent irrigation water is applied annually, irrespective of rainfall which averaged 21.45 inches over the 4 previous years, but totalled 36.07 inches for the year July 1966/June 1967. For 3½ months prior to the outbreak in April 1967, there had been 20.5 inches of rain. The pasture in question had not been grazed for 10 weeks, during which period there had been marked growth and seedhead formation, especially of the summer type *Paspalum* grasses, which had become heavily infected with *Claviceps paspali*.

On the first day of grazing by the heifer herd there were two peracute deaths. Both were reported to have shown nervous derangement before death. No further cases were observed on day 1. On day 2 nothing unusual was observed until late afternoon when five animals were reported ill. On the morning of day 3, 20 animals showing symptoms were observed in the overnight camp and grazing of the affected pasture was withheld.

Later in the day, 12 more had become affected and another five cases were observed on the morning of the fourth day, resulting in a total of 37 clinical cases.

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(Paper presented at the 62nd Annual Congress, S.A.V.M.A., Durban 1967).

SYMPTOMS

All stages of nervous derangement were seen, varying from mild involuntary muscular trembling and slight ataxia to gross inco-ordination of movement with complete prostration.

Some animals appeared normal at rest, but when driven showed a stiff jerky goose-step-like forelimb movement and ataxia of the hindquarters with trembling of individual muscle groups. Others had a very alert appearance and showed severe spasmodic muscular trembling varying from involuntary muscle group tremors and nodding of the head, to shaking of the limbs and trunk. Inco-ordination of muscle groups produced varying degrees of ataxia from stiff-legged jerky movements, sideways progression, and falling, to prostration with inability to stand even when assisted. Of five animals so affected, three resembled typical heartwater with lateral recumbency and violent leg paddling. The other two adopted sternal recumbency and together with the less affected animals retained their appetites.

On day 3, two animals could not rise and on day 4 an additional three were similarly affected. That evening, two of the five got up. The other three were able to rise by noon on day 5 from which time there was a dramatic improvement in all cases although some still showed varying degrees of inco-ordination lasting a few days. No new cases were observed.

POST MORTEM EXAMINATION

Autopsies were performed on the two cases which died peracutely. No microscopic lesions of significance were observed although both animals suffered fairly extensive liver damage due to infestation with *Fasciola hepatica;* this could have contributed to their rapid deaths.

SPECIAL DIAGNOSTIC PROCEDURES

(1) Chemico-pathological analyses, according to described methods[7], were performed on blood of four of the severely affected animals (Table 1).

(2) Toxicity trials were carried out at Onderstepoort on two bovines and two sheep. The animals were bled periodically for analyses.

TOXICITY TRIALS

Green and ripe *Paspalum* seeds, the majority visibly infected with the sclerotial stage of *Claviceps paspali*, were harvested and minced.

Experiment No. 1

An 18-months old Hereford-cross steer was given 386.6 g (2 g/kg. body weight) of the seed per stomach tube.

Slight ataxia and generalised muscular trembling which increased after exercise was noticed on the following day. Symptoms were still present on the third day but absent on the fourth day.

The results of the chemico-pathological analyses are given in table 2.

Experiment No. 2

An 18-months old Hereford-cross steer received an initial dose of 105 g (0.5 g/kg) with no detectable symptoms. It was dosed again on the third day with 1.5 g/kg. On the fourth and fifth day slight ataxia and muscular trembling of the large muscle groups of the hind-quarters were noticed. The animal was dosed again on the seventh day with 3 g/kg of the seed. Apart from ataxia and muscular trembling the animal was also now slightly hypersensitive. A softening in faecal consistency was also observed. On the eighth day 6 g/kg was dosed

Table 1—FIELD CASES

Case:	1	2	3	4
S.R.—Sedimentation rate (mm/hr)	1	1	4	1
P.C.V.—Packed cell volume (%)	36	47	36	50
Hb.—Haemoglobin (g.%)	10.5	13.0	10.25	12.5
S.G.O.T.—Serum glutamic oxalacetic transaminase (King units)	92	200	233	254
S.G.P.T.—Serum glutamic pyruvic transaminasie (King units)	24	144	92	92
S.A.P.—Serum alkaline phosphatase (King Armstrong units)	17.5	17.0	10.5	14.0
B.U.N.—Blood Urea Nitrogen (mg%)	23.9	49.68	34.9	27.6
Sugar (mg%)	54.75	45.5	33.25	77.5

Table 2—EXPERIMENTAL CASE (STEER)

Day:	1	3	4	5
S.R.	2	2	2	1
P.C.V.	41	40	40	43
Hb.	10	9.5	10.5	12.2
S.G.O.T.	92	146	173	112
S.G.P.T.	50	73	125	92
S.A.P.	10	11.0	11.0	9.0
B.U.N.	101.6	18.4	14.7	17.6
Sugar	55.75	72.2	71.5	87.5

and the animal went down during the night and became bloated. On the ninth day the steer could not rise without assistance. When down, the hind-legs were kept fully extended and marked generalised muscular twitchings with fore-leg paddling movements were observed. After the animal had been assisted to stand, its general condition improved slightly. From the tenth to the twenty-seventh day it was unable to remain in a standing position even when assisted; nodding movements of the head and a mucoid diarrhoea were noticed on the tenth to the fourteenth day in addition to the muscular trembling and hypersensitivity. From the fourteenth to the twenty-seventh day no symptom apart from generalised weakness was noticed. No sign of anorexia was noticed during any stage.

Chemico-pathological changes are summarised in table 3. The animal was slightly anaemic at the start of the experiment.

Experiment No. 3

Two young Merino ewes were dosed with 2 g/kg of the seed. Apart from some slight hypersensitivity on the third day, no clinical abnormalities were seen. The chemical pathology of their blood did not deviate from the normal during the course of the experiment.

CONTROL MEASURES

In an attempt to render the pasture safe, affected areas were mown with a rotary type cutter which either cut off or shattered the seed-heads of the high growing *P. dilatatum*. Due to the presence of rocks in the pasture it was not possible to cut the low growing *P. distichum*, which however was not nearly as prevalent at *P. dilatum*.

Once the pasture had been momed, animals were re-introduced on a controlled grazing time basis and closely observed, the grazing time being increased from 2 hours to 4 hours and then to the full 6 hours. In addition, *Eragrostis* hay was fed in the overnight camps (7½ lb. per animal).

DISCUSSION

The danger of grazing rested *Paspalum* pastures after wet humid summers is shown by this outbreak. Where regular grazing has prevented extensive seeding, the risk appears to be minimal, only three mild cases of *Claviceps paspali* poisoning being observed amongst herds on pastures similarly infected with *Paspalum* but regularly grazed.

Table 3—EXPERIMENTAL CASE (STEER)

Day:	1	2	3	6	8	9	13	20	28	31
S.R.	4	4	4	3	4	3	7	10	12	5
P.C.V.	33	33	34	34	32	34	37	29	24	24
Hb.	8.25	8.75	9.5	8.75	8.25	8.5	11	8.25	7.25	7.25
S.G.O.T.	99	119	132	119	112	194	274	166	105	112
S.G.P.T.	36	73	73	30	52	67	79	92	48	52
S.A.P.	10	8.0	9	12	10	11	20	5	6	3.5
B.U.N.	20.2	17.6	17.6	18.4	14.7	17.6	17.6	20.2	17.6	17.6
Sugar	70.25	68.25	49.5	57.75	83.75	91	64	54.7	50.75	54.7

It is noteworthy that there was no loss of appetite, even in recumbent animals, and that rapid recovery followed removal from the infected pasture. The low mortality rate is also significant and tallies with the findings of others.

Following re-introduction to the mowed pasture, no further cases were observed, the majority of the infected seedheads having been removed. Re-seeding of the *Paspalum* grasses was suppressed by the onset of winter.

The elevated serum glutamic-oxalacetic and -pyruvic transaminase levels presumably indicate muscular degeneration and liver damage. The elevated serum alkaline phosphatase is also indicative of liver damage which, in the field cases, may have been clouded by fascioliasis.

The toxicity trials indicated sheep to be less susceptible than bovines.

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INFECTIOUS DISEASES OF CATTLE AND SHEEP UNDER INTENSIFICATION

F. B. W. DU CASSE*

In order to find out just what infectious diseases occur under intensive conditions in Natal, records were obtained from reliable dairy farmers. All their herds were of high quality, kept intensively and reared almost exclusively on artificial pasture. The animals were all T.B. free, inoculated against most diseases for which vaccines are available, milked by machine, the milk production recorded; they were dewormed regularly and fed on modern scientific principles. Half were Jerseys and half were Frieslands, half were inseminated and half were hand-served, a little over half were pedigreed, and all were subjected to regular veterinary supervision. To keep the balance, herds were chosen that were cared for by four different practitioners.

Table 1 summarises the more important conditions encountered in these herds during an average year:

Table 1

Bloat	182 cases	25%	of total
Mastitis	162 cases	22%	do.
Endometritis	130 cases	18%	do.
Foot-rot	78 cases	10%	do.
Milkfever	45 cases	6%	do.
Redwater	43 cases	6%	do.
Digestive disturbances	30 cases	4%	do.
Acetonaemia	16 cases	2%	do.
Miscellaneous	54 cases	7%	do.

Other speakers have, or will be, dealing with many of these problems, and my contribution is therefore limited to mastitis, foot-rot and other such infections.

Mastitis appears to be the most important infectious condition of the modern dairy cow. In my experience, and according to many published reports [1-3] the incidence of this disease is associated with improper milking machine hygiene or function. The almost complete ignorance of the majority of farmers — and of many members of our profession — in regard to the important basic principles of milking machine hygiene and maintenance, is in my opinion, one of the most important contributory causes to this state of affairs. It is evident that future practitioners in intensive dairy areas will require a sound knowledge of milking machines and that air flow meters and the like will have to form a basic part of their equipment. Parker [4] sums it up rather aptly when he remarks that "in advisory work for the past 16 years, investigation and correction of faults in milking on individual farms have been more important than bacterial tests in the laboratory".

Regarding the organisms causing mastitis, table 2 shows that pathogenic staphylococci were the most important.

Table 2

Pathogenic staphylococci	40%
E. coli	26%
Streptococcus agalactiae	12%
Sc. dysgalactiae	13%
Sc. uberis	4%
Corynebacterium spp and others	5%

These figures are to some extent in agreement with the findings of van den Heever & Giesecke [5] except that there is apparently a much lower incidence of *E. coli* infections in the Pretoria area.

Our results of immunisation against staphylococcal mastitis have been most uninformative. In two herds, complete and satisfactory control was achieved, while in four others, no obvious response was obtained. Nevertheless, reviews by Derbyshire [6] and Cameron [7, 8] record encouraging results which

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appear to make further trials warranted. van den Heever [9] on the other hand, contends that high antitoxin titres simply minimise the effect of the toxin on the tissues but will not reduce the incidence of infection. In this respect Parker [4] records that infection often seems to mean the mere finding of bacteria in milk, but that with staphylococci it is difficult or impossible to interpret their significance unless associated with clinical evidence of disease. Conflicting reports of response to immunisation may thus be associated with the workers interpretations of "infection". Nevertheless van den Heever *et al*, record that in a survey of 40 herds involving 2,800 udders and based on microbiological — cytological examination of samples, only 28.2 per cent of udders could be classified as normal while 26.1 per cent were severely diseased — ample support for the contention that mastitis is the dairyman's public enemy number one.

Endometritis and associated conditions were responsible for 18 per cent of disease conditions. Under this heading is included abortion and retained after-birth as the latter, while rather infrequent, generally result in genital infection requiring treatment. Table 3 reflects the laboratory findings in respect of organisms isolated from vaginal swabs or from the genitalia at slaughter.

Table 3

Number of samples	130
E. coli	39 — (30%)
Staphylococci (pathogenic)	31 — (24%)
Mixed	20 — (16%)
Negative	40 — (30%)

The high proportion of negative samples indicates the possibility that viral agents may have been implicated. The exact significance of *E. coli* in genital samples is difficult to assess as they may frequently have been mere secondary invaders or contaminants. In no single case were corynebacteria isolated, whereas in Denmark, Thygesen [10] isolated these organisms in 91 per cent of 700 bovine genitalia at slaughter, suggesting that it was an important cause of infertility in bovines. On the other hand, from 102 foetuses submitted to the Allerton Laboratory in 1966, corynebacteria were isolated in 14 per cent and incriminated as the cause of the abortion. This organism does thus appear to be of significance in bovine genital infections. The detailed findings in regard to foetuses submitted are reflected in table 4.

Table 4

Number of foetuses	102
Corynebacteria	14%
Brucella	4%
Vibrio	4%
E. coli	4%
Fungi	2%
Negative	74%

Footrot was the next major cause of concern. It was far more prevalent in Frieslands than in Jersys and occurred almost exclusively during the wet summer months. The term "footrot" is used but strictly this is incorrect as the aetiology of many cases was not established. Nevertheless, the figure of 10 per cent for our conditions is very similar to the 9 per cent recorded by Amstutz [11] in America. This writer also found that lameness was the most important pathological condition of dairy cows after diseases of the reproductive tract and udder, and considered that infectious pododermatis was a major cause of such lameness. Gould [12] referred to the importance of lameness in cattle in the United Kingdom, but considered that less than 50 per cent were caused by footrot, the majority being due to soft horn, penetrations by foreign bodies, bruising and other injuries. Fritsch [13] recorded that 6-8 per cent of cattle were found to be suffering from diseases of the hoof and considered that this percentage could be regarded as an average of the incidence of diseases of the feet of milk cows in Germany. Whatever the exact percentage of lameness caused by footrot may be, this disease is undoubtedly of considerable economic importance under intensive conditions.

Babesiosis or redwater was responsible for some 6 per cent of the recorded disease conditions. While this disease is a very important problem on many semi-intensive and most extensive farms in Natal, it was somewhat unexpected in cattle on artificial pasture. Enquiries revealed that two severe outbreaks had been experienced, both associated with veld hay provided to stock at pasture as a bloat control measure; this hay proved to be tick-infested. Arthropod-borne bovine diseases were otherwise of little importance.

Under the heading **"miscellaneous"** were 54 cases, representing 7 per cent of the total. These included two cases of navel-ill, four of

suspected calf diptheria, and some 16 classified as undiagnosed, with an occasional suspicion of leptospirosis.

The exact significance of **Leptospirosis** in cattle in South Africa requires further research. We have serological evidence of the disease on eight farm in the Midlands of Natal — with titres as high as 1 in 12,000. Table 5 reflects the incidence encountered in this area:

Table 5

Number of herds	13	Positive	8 (62%)
Number of cows	116	Positive	21 (18%)

This coincides closely with the infection rate of 17.1 per cent recorded by Botes *et al* [14] in the Orange Free State, but is well below the level of 40 per cent infection reported by Michna [15] in British cattle. The F.A.O. Expert Committee on Zoonoses [16] contended that leptospirosis constitutes a major problem in cattle, so this is possibly a disease we shall encounter more frequently in the future.

Regarding **Tuberculosis,** table 6 shows the incidence in dairy cows on eight farms in the Natal Midlands, and indicates the magnitude of the problem when cattle are kept intensively.

Table 6

Number of herds	8
Number of cows	1053
Number positive	421 (40%)

Johne's disease is, as yet, rarely encountered in the Republic. Clinical cases occurred on a farm in this area and some 20 per cent of the herd was subjected to serological and skin sensitivity test. An interpretation of the results is reflected in table 7. So far, none of these 20 reactors have shown any clinical evidence of infection; perhaps the disease will not prove a problem in this country. On the other hand, intensive husbandry in the cool wet areas may prove suitable for outbreaks. The report by Lovell [17] of the spread of the disease in Iceland after the introduction of infected sheep, and resulting in the death of 75,000 to 100,000 sheep in 15 years, gives food for thought.

Table 7

Number tested	58
Positive	20 (35%)
Suspicious	13 (22%)

While *Clostridium oedematiens* has not been recorded as an important pathogen of stock in South Africa, it does occur. It is a well known cause of sheep and cattle mortality in Australia, and Williams [18, 19] emphasised its significance as a cause of sudden death in sheep and cattle in Wales. Batty *et al* [20] found that it was quite a common infection in sheep and cattle in Great Britain, and in 1967 [21] they recorded *Cl. oedematiens* in 31 out of 232 smears from cattle dying in South Africa. The same investigators [20] suggested that, as an alternative explanation to the well-documented role played by migrating flukes in activating latent *Cl. oedematiens* spores, metabolic disturbances resulting in liver damage may allow this organism to proliferate and produce toxin, thus causing some of the many unexplained stock deaths encountered. If this supposition is correct, it is possible that this disease may assume an increasingly important rôle on farms in South Africa where intensive livestock husbandry is practised. Only when fluorescent antibody staining technique facilities for the simple and rapid demonstration of specific bacteria in smears from tissues and for the typing of pathogenic organisms are available at local diagnostic centres, will it be possible to establish the exact significance of this organism as a cause of stock losses in the Republic of South Africa.

With regard to **sheep,** I have been unable to obtain any reliable statistics of disease conditions on specific farms. The big problems would appear to be verminosis, footrot, peri-natal mortality, vuilbek (orf) and lumpy wool. In 90 cadarers autopsied at this laboratory during one year, verminosis was diagnosed in 68%, enterotoxaemia and domsiekte each in 5.5%, and bluetongue and pasteurellosis each in 3%. In regard to peri-natal mortality, table 8 reflects our findings to date:

Table 8

Number of autopsies	80
Coli bacillosis	57—71%
Others	23—29%

We are not, as yet, sure of the aetiology of "others". There is a suspicion of **Listeriosis** but this awaiting confirmation. This disease has been recorded in chinchillas in South Africa by du Plessis *et al* [22], but not as yet in the sheep.

Toxoplasmosis is another possibility. In South Africa it has been recorded by Smit [23] in dogs, Bigalke *et al* [24] in ferrets, and du Plessis *et al* [25] in chinchilas, so there is reason to suspect that it may be playing a rôle in sheep. Smith [26] certainly considered it a major cause of ovine abortion in Australia and New Zealand, so it is a disease we are on the look out for here.

Colisepticaemia does however appear to be the major cause of lamb losses. It has been reported from various sheep raising areas of South Africa by Botes [27], in Australia by Roberts [28], and in New Zealand by Kater *et al* [29]. Our experience would tend to confirm the views of Botes [27], the available evidence indicating that the incidence of this highly fatal disease is increasing.

We have encountered **Pasteurellosis** in lambs, especially during spells of cool weather when grazing on low-lying moist pastures. Removal to drier pastures has tended to prevent mortality.

Vuilbek, (orf) is at times a serious problem especially when first encountered on farms. Under intensive conditions, spread is very rapid and frequently morbidity is 100% before an autogenous vaccine can be prepared and used.

Lumpy-wool, during wet years, tends to play a rôle in mortality of young lambs, but in normal seasons is not of much practical significance.

To summarise table 9 shows the most important infectious disease problems encountered in intensive livestock units in Natal during the past 18 months.

Table 9

	E. coli	S. aureus
Mastitis	26%	40%
Endometritis	43%	35%
Lamb mortality	70%	—

Pathogenic staphylococci and *E. coli* stand out as the major pathogens and would appear to represent a serious challenge to our profession if we are to keep pace with disease control under conditions of increasingly intensive livestock husbandry.

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NUTRITIONAL DISEASES ASSOCIATED WITH INTENSIVE PRODUCTION

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INTRODUCTION

The tendency in animal production is towards a concentration of animal units with corresponding increase in productivity per unit area of land which is desirable where space is limited and land prices are rising. The primary cause of the re-evaluation of production concepts, however, is the resultant rise of returns on capital investment which, under optimum conditions, compare favourably with returns from similar invested capital in other industries.

The confinement feeding of animals was practiced first in the pig and poultry industry; it has not been limited to these species, however and the feedlot systems for ruminants are here to stay. One important reason for the current surge to intensive feeding methods is the favourable price-ration between grain and meat in the Republic.

Producers of beef, mutton and milk in parallel ways have intensified production methods, with the incorporation of high yielding heavily fertilised forage crops in their feeding programs. These crops are capable of consistently high yields which only a few years ago were considered unattainable or otherwise uneconomical.

An accelerated production tempo necessitates frequent evaluations of the production methods so as to assure the maximum efficiency in the utilization of the large volume of feed ingredients which are processed through animals. An aspect which has received attention with the increased turnover rate, is the possible application of mechanisation in the large production units. This in turn influences management, reducing labour requirements but increasing the required skill. Marketing methods are also scrutinised as profits are at times lowered in anticipation of the increased total profits resulting from the faster and bigger turnover rate.

There are several stages of intensification between the extensive production systems and the sophisticated intensified feedlot, hence the problems which are recognised may be classified in groups according to the stage of development.

RANDOM SUPPLEMENTATION

Extensive production systems rely on natural grazing as the sole source of nutrients. This supply fluctuates seasonally in quantity and quality and frequently from year to year. It is logical therefore, that efforts be made to reduce these extreme variations by supplementing nutrients. This supplementation should be based on analytical data.

The necessity for specificity is illustrated by the recent investigation conducted into a condition of sheep in the southern part of the Orange Free State. As a result of consecutive dry years in the area, emphasis was placed on supplementary licks.

In early 1967 a condition was referred to the Veterinary Research Institute by Veterinary Field Services which clinically appeared as an afebrile stiffness in 5-10 per cent of the adult sheep which were in poor condition. Necropsies revealed a generalised mineralisation throughout the blood vessels. Broad spectrum mineral analysis of samples indicated that the primary condition was a calcification of the soft tissues which possibly was precipitated by a magnesium deficiency. Unassociated with the primary condition was a manganese deficiency which also warranted supplementation. It is apparent that large numbers of sheep received licks, at considerable expense and effort, but despite the

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good intentions the random approach to the formulations produced disappointing results.

In this, the first stage of intensification, it is necessary to conduct limited well-planned investigation which, with the necessary broad spectrum analysis made possible by atomic absorption spectroscopy, will crystallise the correct recommendations for a specific area.

ANTAGONISMS

The supplementation of trace elements and minerals necessitates the recognition of other minerals which are associated with them. This is not only of importance in the formulation of animal licks but also when pastures are to be fertilised. In the Republic sulphur is frequently included in lick formulae at levels reaching 7 per cent and higher which may precipitate a selenium deficiency by increasing its excretion. Such an induced deficiency may also be precipitated by the use of sulphur-containing fertilisers, such as gypsum, which reduce the uptake of selenium from the soil by the plant [1, 2, 3, 4]. When selenium is marginally adequate it is possible to precipitate degenerative myopathy in sheep by sulphur supplementation [5]. Conversely sulphur can be used to suppress selenium uptake in areas in which toxic levels occur.

An important consideration is the plant-animal relationship. The mineral requirements of plants and animals do not entirely correspond either qualitatively or quantitatively. Administration of molybdenum to pastures often produces dramatic responses in plant growth but it is known that copper and molybdenum are antagonistic in the presence of sulphur [6]. If, therefore, copper is only marginally adequate in the soil a deficiency will be induced in the animal. Elevated copper and sulphur levels can, however, be used beneficially where molybdenum levels are high. A further interesting assiciation [7] is the possible blocking of the antagonistic effect of molybdenum on copper by the elevated manganese level.

The use of macro-elements in fertilising programmes boosts forage yields and usually raises the nitrogen content of the plant material, thus changing the ratio with the related minerals. Administration of 50 compared to 300 pounds of ammonium nitrate nitrogen per acre raised the nitrogen content of the dry forage by 71 percent. Associated with the rise in nitrogen was an increase in the retention of calcium and potassium but a 62 percent drop of available magnesium, thus decreasing the magnesium balance [8]. It becomes apparent that a hypomagnesaemia and tetany [9] may be induced by emphasizing nitrogen fertilisation when magnesium is only marginally adequate.

VITAMINS

Guilbert and Hart [10] first established the vitamin A requirements of beef cattle and since then there have been repeated re-evaluations of the concentrations required by beef cattle on intensive feeding programs. A synergistic relationship has also been recognised between vitamins A and E. Animals on winter grazing or in feedlot systems are generally known to benefit from the supplementation of 25,000 i.u. of vitamin A daily [11]. The benefits noted include increases in daily gain and feed consumption as well as an improvement in feed conversion [12].

The presence of vitamin A antagonists has been recognised, and it has been proposed that factors which block the reticulo-endothelial system reduce the vitamin A level in animals by preventing the deposition of vitamin A ester in the liver [13]. Disease of an infectious nature, particularly those affecting the liver, place an increased drain on the body stores of this vitamin.

Symptoms of vitamin A deficiency have appeared in feedlot cattle when known quantities of vitamin A were being supplemented in excess of 20,000 i.u. daily. The symptoms of brisket oedema, nightblindness and reduced feed efficiency could be alleviated by either elevating the vitamin supplementation rate beyond 40,000 i.u. of A or by adding vitamin E. From these observations it may be concluded that the utilisation of vitamin A was being interfered with and that vitamin E with its anti-oxidant action was capable of preventing the interference. The nitrate content was comparatively high in the silage being fed in this specific case, but there is no conclusive evidence that this precipitated the condition. It has been noted that urea could reduce the liver storage of supplemental vitamin A in sheep, but these results could not be confirmed in a subsequent series [14].

Recognition of the actions of synergists and antagonists in vitamin metabolism necessitates re-evaluation of our concepts of vitamin interactions and requirements.

CONCENTRATES

It is traditional to associate ruminant feeding with the utilisation of considerable volumes of roughage in the range of 50-75 per cent of the total feed intake. Recent evidence indicates that the feeds used most efficiently by growing finishing cattle are those which are fermented in the rumen to produce more propionic acid and less acetic acid [15]. The production of propionic acid increases when starch in the diet is increased hence it is a logical conclusion that heavier grain rations deserve consideration, but certain problems are associated with such rations. These include frequent bloating, vitamin A deficiency and calcium inadequacy. It appears that a minimum coarse roughage content in the diet of 25 per cent avoids these disturbances.

Since 1960 grain feeding has been emphasised in Britain by the use of rolled barley as the main ingredient in the range of 85 per cent of the total the remainder being protein, mineral and vitamin supplements. Disease problems were not encountered despite the absence of separate roughage but attention was given to the method in which the grain was processed so as to retain the roughage characteristics of the husks [17]. In lamb feeding experiments where fibre was not included in the ration, it was found that satisfactory growth was obtained provided that diet was supplemented with sodium and potassium salts of the lower volatile acids [18, 19]. Use of ground maize and protein supplement with only 2.5 per cent fibre and 2.5 per cent sodium bicarbonate in cattle experiments, however, resulted in severe bloating and a 33 per cent mortality [20].

The use of neutralising agents in low-fibre diets has not given consistently beneficial results. It is evident that limited roughage in one or other form in high grain rations gives more consistent benefit.

The discussion, so far, has been orientated towards the barleybeef animal which is included in the intensive feeding programme from three months of age and has therefore never received large quantities of roughage. It is possible, however, to introduce animals to intensive feeding after a period of roughage feeding as is the practice in the majority of feedlot systems in the U.S.A. If such a transition is too rapid, posterior paresis, dullness and anorexia may develop and several of the animals may go down. These symptoms are typical of acidosis caused by the high rate of starch intake and its conversion to lactic acid. This does not only occur in the unadapted animal, as "downer" cattle are occasionally also found in the feedlot during the terminal stages of high grain feeding shortly before marketing. There is no practical remedy for these individual animals, as their metabolic pathways cannot cope with the load placed on them and it is the practice to market them immediately before there is a marked loss in condition.

The feeding of high levels of grain is also the cause of deviations from normality which are not usually recognised in the live animal. At slaughter it is found that liver abscesses develop in as many as 25 per cent of the animals in intensive feedlot feeding. The carcase is otherwise unaffected. It has been suggested [21] that the abscesses develop as a result of the lowered pH with resultant damage to the ruminal mucosa permitting the introduction of pyogenic organisms which localise in the liver. Kidney necrosis also occurs to a considerable extent but often passes unnoticed at the abattoir. Changes in the rumen vary from parakeratosis to ulcerations of considerable magnitude. The most extensive ulcers observed so far have been in fistulated steers receiving 90% grain rations. The ulcers were in excess of 30 cm in diameter and were situated on the pillars subdividing the rumen.

PROCESSED FEEDS

The efficient formulation of high-production rations necessitates primary consideration of local production of suitable ingredients so as to ensure practical and economical formulations. There is, however, considerable scope in varying the forms in which ingredients are fed so as to improve acceptability and conversion efficiency.

In ruminant nutrition attention has been given to the various physical forms in which hays can be fed. Amongst the newest developments is the wafer which can be made from whole, chopped or milled hay and was introduced in 1961 [22]. Wafers 6.4 by 5.7 cm of varying lengths were fed to sheep [23], which showed a preference for the wafered over the chopped hay. The wafering process reduced the carotene content of the hay probably as a result of the temperature rise to 50° C. Milling the hay prior to wafering also reduced the digestibility of crude fibre as expected. Nevertheless, no bloat problems were en-

countered as a result of the wafering as has at times been observed with the use of pelleted roughage.

In the pig, ulcerations of the digestive tract are receiving considerable attention and it appears that particle size of the meal, the heating of grains and the presence of fibre are factors closely associated in inducing and preventing oesophago-gastric ulcers. A wide range of supplements has been used in efforts to control the incidence including antibiotics, water and fat soluble vitamins as well as proteins [24, 25], but without success. The inclusion of oats and wheat however, resulted in a significant decrease in ulcerated stomachs and this is attributed to the fibre.

Investigations into the effect of the particle size of the ration revealed the interesting fact that more lesions develop as the milling of the maize and other ingredients becomes finer [26]. Furthermore, the use of "expanded" maize (maize processed by heating) also aggrevated ulcer development. A recent paper [27] also incriminates the pelleting process as the cause of ulcers. Remilling the pellets before feeding did not avoid the development of the oesophago-astric ulcers, thus permitting the deduction that the heating of the ration, which is inevitable in the pelleting process and which is associated with the "expansion" of maize, brings about chemical changes in the feed ingredients which are conducive to ulcer development.

In the above paper it is also stated that the pelleting process, despite its ulcer producing effect, improves feed efficiency both in the pelleted and in the remilled form. This explains why so much time is spent in manouvering animals and their feeds on the fringes of normality. The field of research into nutritional abnormalities is so extensive as to stimulate most imaginations, so productive as to encourage research and remunerative when the results are of direct significance in animal production.

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SWINE HEALTH UNDER INTENSIFICATION

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In the annual report of the Livestock and Meat Industries Control Board for the year ended June 30th 1966, pig slaughterings showed a remarkable increase of 18%, and, for the first time, more than one million pigs were slaughtered in South Africa in a calendar year. The eastern and western maize regions comprise the most intensive pig production areas, with the greatest increase in production occurring at the moment in the western Transvaal.

Much of this increased production comes from the rapid expansion and intensification of existing piggeries, often without sufficient buildings being available for the extra stock now being kept. The largest commercial piggery in S. Africa known to me comprises 705 breeding females and their followers, a total of some 3,000-4,000 pigs concentrated in an incredibly small area. Numerous 300-500 sow units are now to be found in the main production areas, and many more may be expected. Intensification is definitely a fact in the pig industry and we must be prepared to offer the technical guidance required to maintain the health and productivity of such large units.

In giving such advice, we are often gravely hampered by the inadequate and overstocked housing to be found on many of the older farms. Here no compromise is possible and one should be ruthless in condemning the monstrosities which have passed for pig houses in the past. The key to effective health control is to be found in correctly designed and constructed housing. While most fattening accommodation is still of the climatic variety — and usually far too big — controlled environment farrowing housing, complete with temperature control and adequate anti-crushing devices, is at last making its appearance. The sow is particularly sensitive to high ambient temperatures at farrowing time, and the ventilation and insulation of the farrowing house must be such that air temperatures inside the house do not exceed 75° F.

Weaned pigs are best housed in litter groups, thus eliminating the exchange of infection at this susceptible age and reducing the stress-induced diseases, such as oedema disease, to a minimum. One-stage housing, with the housing of a litter from birth to marketing in one all-purpose pen, is being tried and certainly has many favourable epidemiological and managerial implications. If one considers that adenovirus infection has recently been reported to be widespread in British pigs[1] it seems obvious that the large weaner groups, so prevalent on many farms, may serve to introduce all sorts of undreamt-of agents into highly susceptible populations. In all farrowing and fattening accommodation, the provision of numerous small units must be envisaged, where periodic depopulation and disinfection — the "all in-all out" principle — can reduce that poorly understood hazard we call "disease build-up" for want of a better name.

The reproductive life span of our sows appears to be on the short side. Joubert has reported[2] that the sows culled from the Pretoria University herd were just under 3 years old and had produced an average of 3.2 litters. While all were not culled for infertility or ill-health, this figure does not compare favourably with those obtained in two British surveys[3,4] which yielded figures of 3.7 and 3.75 litters per culled sow respectively.

While there is still a great deal to be learnt about the underlying causes of porcine reproductive failure in all its forms, certain useful information has lately become available which emphasises that sow groups should be kept small, ideally about 12 sows together in a yard, and provided with suf-

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ficient shade and individual feeding facilities. Recent American[5] and British[6] reports have described a viral cause for stillbirth and abortion, and Texan workers[7] have isolated a PPLO capable of producing toxic agalactia. They claim that this infection is probably spread by nose to nose contact. In this country subclinical attacks of erysipelas and leptospirosis may cause abortions, while eperythrozoonosis is becoming disquietingly prevalent in some herds.

Stress at weaning seriously disturbs the bacterial equilibrium of young pigs. The main stresses appear to be movement, severe temperature fluctuations (which are usually aggravated by inadequate housing) and overfeeding with protein. Clinically, the setback is manifested as a diarrhoea, usually associated with one of the enteropathogenic *Escherichia coli* serotypes or as the far more serious vibrionic swine dysentery. Ducasse and Nixon[8] have recently emphasised the rôle of excess protein feeding in exacerbating this disease.

Gross overstocking leads to bullying, savaging and tailbiting, followed by vertebral abscessation and paraplegia or possibly spirochaetal abscessation and severe lameness. Salmonellosis, generally due to *S. typhimurium*, also occurs from time to time in such overcrowded groups, but appears to be far less prevalent than a few years ago. Enzootic pneumonia has unexpectedly become difficult to find in our commercial herds and can no longer be regarded as of serious economic significance in this country. Climatic housing must probably be given the credit for this pleasant development.

It may surprise you to learn that tuberculosis has become one of the major sources of economic loss to the pig producer in recent years. The bacterial typing of organisms cultured from the lymph nodes of many of these animals has revealed the unexpected fact that some 80 per cent of these pigs are now found to be infected with an atypical mycobacterium known as an aviumlike organism, or as *M. intracellularis*. At the present, the source, reservoir and epidemiology of these organisms is unknown[9], and one is in the frustrating position of being unable to offer any worthwhile advice to harassed producers who may be losing as many as 5-8 per cent of each consignment to the abattoir being condemned for generalised tuberculosis. This problem is also occurring in many other countries, and no solution to the enigma of the origin of these organisms is apparently in sight.

Other disease problems which are gradually becoming more prevalent under intensified husbandry are gastric ulceration and the sudden death syndrome known as "red gut" or "intestinal haemorrhage syndrome". While oesophago-gastric ulceration of the non-glandular cardiac area of the stomach has been seen in association with the acute liver dystrophy of both *hepatosis diaetetica* and copper poisoning, the cause of primary gastric ulceration, unassociated with liver disease, remains unknown; the incidence however is probably increased by stress. Jones[10] has suggested that "red gut" may be associated with an allergy to milk proteins, but the disease, commonest in baconers, has often occurred on farms not feeding milk products.

An attempt has been made to discuss in broad outline some of the philosophy underlying modern concepts of health promotion under intensive swine husbandry conditions.

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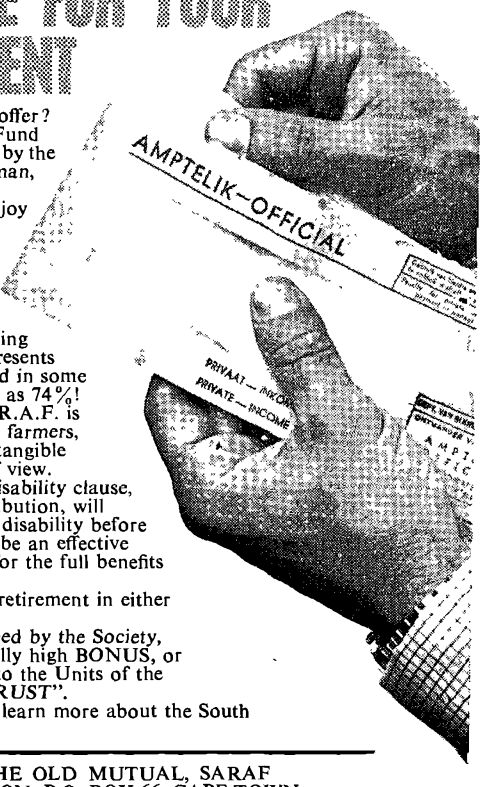

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RESUME OF UNPUBLISHED PAPERS PRESENTED TO THE 62nd CONFERENCE OF THE SOUTH AFRICAN VETERINARY MEDICAL ASSOCIATION HELD IN DURBAN, 1967

PROBLEMS ASSOCIATED WITH DISEASES OF POULTRY UNDER CONDITIONS OF INTENSIFICATION

I. VAN SCHALKWYK*

The explosive growth of the poultry industry over recent years has created many problems most of which can be grouped under the following headings:

1. **Failure to prevent the spread of disease**

This is partly due to gaps in our knowledge of the epizootology of many diseases but often due to ignorance or dishonesty. For instance, breeders of day-old chicks who are holders of disease free certificates will often when pressed, obtain eggs from any available source. Elaborate precautions are often taken against the introduction of disease by some routes while others are left wide open. Stricter control and wider education of poultry farmers are required.

2. **Inadequate diagnostic facilities**

The speaker could not envisage adequate diagnostic and expert advisory services being supplied by the State alone. The poultry industry must first realise the need for such expanded facilities and then find means of supplying them themselves. It was also suggested that the handing over of vaccine production to private enterprise should be considered.

3. ***The fact that most poultry plants in South Africa kept several ages of birds on the same premises***

This was due to the fact most plants were relatively small and had been built up to supply a local limited market. The constant introduction of day-old chicks into premises where older birds were housed exposed the chicks to endemic diseases and made vaccination programmes difficult. Integration of the industry into larger plants each dealing with specific age groups was an inevitable development.

4. **Conservatism**

Many poultry farmers were slow to introduce many scientific practices and also resisted the introduction of modern integrated "factory" farms as this took away their independence. In the speakers opinion they would have to accept such changes or go to the wall.

* P.O. Box 26, Hammarsdale, Natal.

TOXICOLOGICAL PROBLEMS IN RUMINANTS UNDER INTENSIVE FARMING CONDITIONS

T. F. ADELAAR*

Among the conditions encountered on cultivated grass pastures were mentioned hydrocyanic poisoning on *Sorghum* and *Cynodon* species, photosensitisation on *Panicum* species and *"Phalaris* staggers". This last condition occurs when animals have grazed exclusively on *Phalaris* for some weeks. They show inco-ordination and hyperexcitability. The condition can be prevented by dosing cobalt but it is not a cobalt deficiency.

"Fescue foot" is a necrosis of the extremities with sloughing of the hoofs, tail and sometimes ears. The symptoms resemble ergot poisoning but the condition is not caused by a fungus.

"Rye-grass staggers" may be caused by excessive ammonia in the rumen interfering with magnesium absorption. Wheat pasture poisoning and rickets in lambs on green grazing were also mentioned.

Nitrate poisoning may follow heavy nitrogen applications to pastures. The nitrate is

reduced to nitrite in the rumen and this may produce methaemoglobinaemia. The diphenyl-amine field test for nitrates is very useful.

Among the fodder crops *Brassica oleracea* (Marnonstem kale) causes acute pulmonary emphysema and oedema, atony of the forestomachs, constipation, blindness, dementia and haemoglobinuria. Sometimes liver necrosis and photosensitisation are seen.

Toxicity of concentrates may be due to ricin poisoning where castor oil cake is used trichlorethylene residues where this has been used as an extractive, and gossypol poisoning on cotton seed cake.

SMALL ANIMAL PRACTICE IN THE CITY

J. L. DORÉ

As to be expected, transmissible diseases were rife among a crowded city dog and cat population. Vaccination has done much to control distemper but a large number of dogs are not vaccinated. The diagnosis of nervous distemper has become more difficult with an increase in cases of neurotoxic insecticide poisoning. Toxoplasmosis must also be considered in such cases.

The infectious hepatitis vaccine has also proved very effective. The speaker also pleaded for revision of the show standards for various breeds of dogs along scientific lines. Congenital confirmational faults were being perpetuated to conform to artificial standards which took no cognisance of the principles of anatomy, physiology or genetics.

Worm infestation was heavy among city dogs. Hookworm eggs are frequently found in the faeces of puppies at 18 days of age and those of roundworms at 28 days. Whipworm *(Trichuris vulpis)* has so far been found only in imported dogs and their contacts. The symptoms of infestation are poor appetite unthriftiness and sometimes blood-stained loose stools. The infestation in itself is seldom fatal unless it is very severe but can be serious when complicated by hookworm infestation. Prevention of further importation of infested dogs was advocated.

The incidence of *Dipylidiae* in dogs is very high. The speaker discussed the possible interconnection between this infestation, flea infestation and pruritic eczema and the possible rôle of allergic reactions.

Traumatic injuries due to traffic accidents and fights are extremely common. With regard to cats, infectious feline enteritis and viral respiratory infections are enzootic with periodic epizootics. The vaccine is most effective. Strangely, verminosis is not severe among the cats.

* Veterinary Research Institute, P.O. Onderstepoort.
* 348 Berea Road, Durban.

DIGESTIVE AND METABOLIC DISTURBANCES WHICH MAY BE ENCOUNTERED UNDER INTENSIVE STOCK FARMING CONDITIONS

R. CLARK*

Intensive stock farming implies high production per animal. This in turn demands (a) animals of high production potential which must be capable of a rapid food conversion and a high rate of metabolism and (b) a diet rich in protein and energy but usually low in fibre. The two main digestive disturbances which can be expected are frothy bloat and abomasal displacement. Lack of roughage probably plays a major role in the pathogenesis of both these conditions.

The well known effect of lack of roughage causing a drop in butter fat percentage was discussed and explained.

Secondary ketosis may be common due to the high rate of metabolism. Disturbances in mineral metabolism are common under intensive conditions. These are due to failures in absorption or internal regulation rather than actual deficiencies. Lush green grazing is high in potassium and protein but rather low in calcium. High ammonia levels in the rumen due to the high protein may interfere with the absorption of magnesium, calcium and potassium. The problems of "grass tetany" and "the downer cow" were discussed against this background.

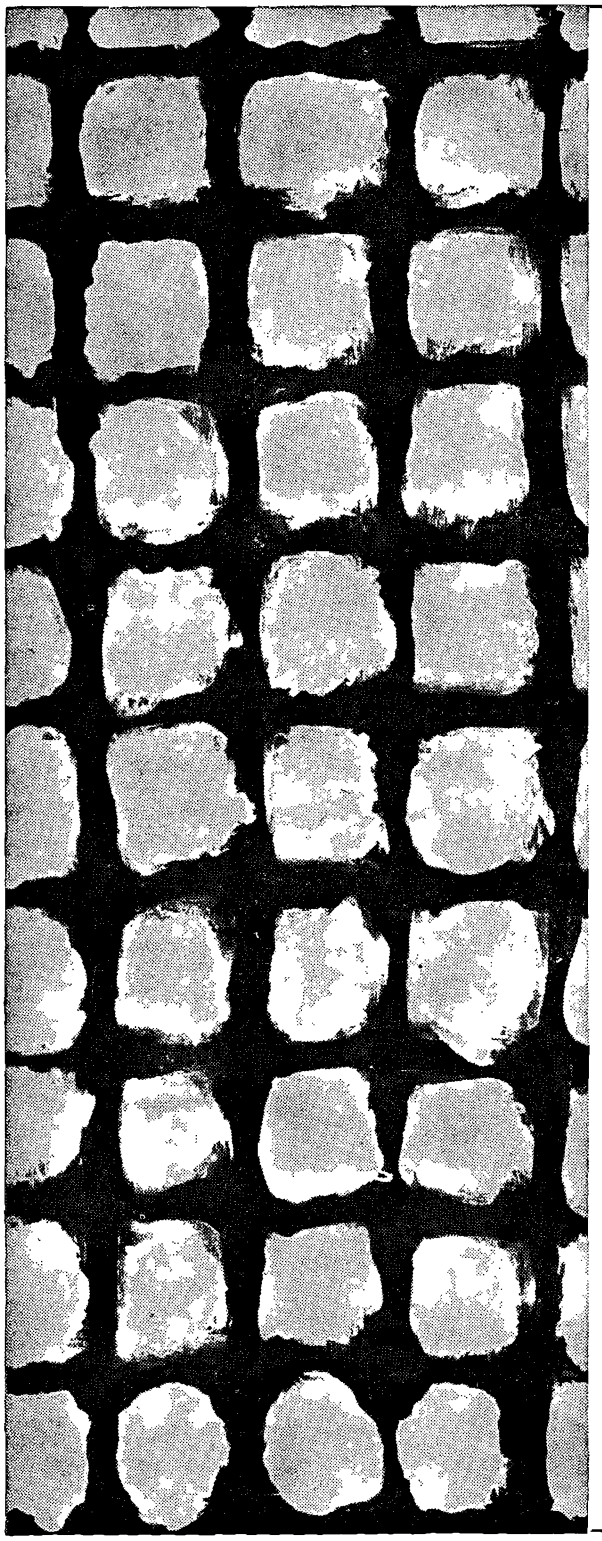
An increased incidence of milk fever can be expected as production per animal rises. This condition is also making its appearance in sheep under intensive conditions. It was pointed out that recent work has shown that "milk fever" is not a simple hypocalcaemia. Hypophosphataemia is actually a more constant symptom.

* Dept. of Physiology, Faculty of Veterinary Science, University of Pretoria. P.O. Onderstepoort.

THE BRITISH EQUINE VETERINARY ASSOCIATION MEETING

DR. J. G. BOSWELL* gave an account of the meeting held at Bristol during July, 1967. The papers mentioned were on the state of training and working capacity of race horses, Cardiology, Iliac thrombosis, Arthritis, Subchondral bone cysts, Virus infections, Anaesthesia, Horse sickness and Artificial insemination. Members interested were referred to published reports of these papers.

* P.O. Sandown, Johannesburg



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BOOK REVIEW

VETERINARY RADIOLOGY

W. D. CARLSON

Second Edition, Philadelphia, Lea & Febiger, 1967. Pp. 666, Figs. 1282, 1 colourplate. Price approx. R22.

The general lay-out of this excellent book remains unchanged but the subject-matter has been expanded, re-arranged and thoroughly revised. Enlarged reproductions have improved the general quality and detail of the radiographic illustrations. The main detraction of the first edition has thus been largely eliminated.

The first part of the book comprises 134 pages and covers all the practical facets of the reproduction of acceptable diagnostic radiographs as well as some of the basic principles of radiological interpretation. The chapter "Basic needs for Veterinary Radiology" has been omitted and a short chapter on the history of veterinary radiology included.

The main part of the work is contained in the second part. In 468 pages a comprehensive and systematic atlas of radiographic pathology for both small and large animals is given. Several improvements have been instituted in the section on small animals, viz

A system approach for thoracic and abdominal radiographic pathology is adopted.

Lesions of the vertebral column are dealt with in a separate chapter.

Normal radiographic anatomy is more extensively illustrated and explained at the beginning of each subdivision.

Special radiographic techniques are no longer dealt with in a separate chapter but apportioned to the respective subdivisions.

Labelled line drawings have been increased from 5 to 68. They cover both normal and abnormal radiographic features and facilitate interpretation of the radiographic reproductions.

The radiological diagnosis of cardiac enlargement, employing straight radiographs, has been considerably extended to include all the latest refinements. A table, stating the ages at which the various ossification centres appear and the epiphyseal lines close, has been incorporated in the chapter dealing with the extremities.

The inclusion of 26 labelled line drawings illustrating mainly the normal radiographic anatomy of the lower extremities considerably improves the section on large animals.

The third part conveys in 37 pages some essential and practical information on the basic principles of radiation therapy and a survey of nuclear medicine. The latter chapter has been entirely rewritten by Dr. Gillette who also collaborated with Prof. Carlson on the chapter on radiation therapy.

The book is properly indexed and adequately referenced for further reading.

In the reviewer's opinion the 51 photographs of surgical and pathological specimens could have been omitted and the space put to better use.

Some illustrations still do not merit inclusion due to poor contrast, unnecessary duplication or lack of independant illustrative value.

The chapter on radiation safety could have been incorporated in the chapter on radiation therapy.

These minor criticisms do not detract from the over-all excellence of this well-produced book.

C. J. R.

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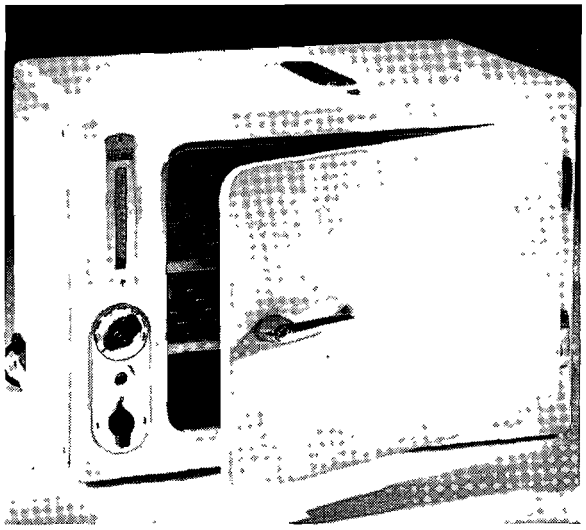

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