Oesophagostomiasis in Sheep (Preliminary Note).

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The life history of *Oesophagostomum columbianum* (Curtice, 1890, Stossich, 1899) was briefly described by the writer in the Ninth and Tenth Reports of the Director of Veterinary Education and Research, 1923. The object of the present paper is to report on some preliminary work undertaken for the purpose of obtaining information on various aspects of Oesophagostomiasis in sheep and can be arranged as follows:—

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I.—The Technique of Artificial Infection of Sheep with *Oesophagostomum Columbianum.*

IA. *Preparation of the Host.*

Previous experience in connection with the rearing of lambs free from worms had shown that several difficulties had to be overcome, e.g., in the first place, climatic conditions came into consideration in the winter months when the weather proved to be too severe for lambs kept under an iron roofed shed, and means had to be devised to enable the lambs to receive adequate air space, and yet at the same time to afford adequate protection against the extreme cold; again, it had been clearly shown that if lambs were allowed to run with their mothers even for a few days only in a bare camp, the lambs generally showed a slight infection of *Haemonchus contortus* or of *Trichostrongylus;* the hand rearing of lambs also presented difficulties, and the utilisation of cow's milk diluted with water resulted in the lambs becoming very unthrifty, and the majority died before the experiment could be brought to a conclusion. Bearing these different points in mind, for the present work a rough and ready cage of suitable size was utilised having a wooden floor and sides of wire netting. The cage was raised from the ground by wooden legs standing on a thick sheet of anthracite, and it was protected from the weather by an iron roofed shed (see Fig. 1); in particularly cold weather dry hay was placed in the cage as additional protection to the lambs which were put into the cage immediately after birth. The wooden floor was washed weekly with disinfectant. The lambs were fed from the bottle with milk from the ewes supplemented daily with a ration of dry autoclaved hay and bran, and about six lambs could be reared at a time. Every fifteen days a sample of faeces from the lambs was cultured with the object of ascertaining whether they were clean. The majority of lambs were kept for twelve months, but several were kept in the cage for more than eighteen months, and during the whole period of examination, cultural tests of the faeces failed to reveal the presence of larvae of *Haemonchus contortus, Trichostrongylus, Oesophagostomum*, etc.

Ib. *Specific Identification of Mature Larvae.*

When sheep run under the usual veld conditions, they are invariably infected with several species of worms, and in order to obtain pure strains of a specific worm infection, it was necessary to devise a method for the preliminary identification of mature larvae. In the previous paper referred to, reference was made to the specific identification of the larvae of the round worms that are most commonly found in sheep in South Africa. These species are illustrated in Figs. 3, 4, 5, 6 and 6A, which latter illustrates the larvae of *Bunostomum trigonocephalum*, also common in South Africa. The tails of the larvae of these five species are shown in magnification in Figs. 7, 8, 9, 10, and 10A. A magnification of 50-70 diameters admits of an easy determination, whilst to the accustomed eye, the identification of the larvae under the binocular can be undertaken with a magnification of 35 diameters. The method adopted for obtaining pure strains of larvae was to culture the faeces of infected sheep in the common fruit jar and to collect the mature larvae from the walls of the jar by means of a piece of moist blotting paper. The blotting paper was then washed in a few c.c. of water in a watch glass, and under the binocular at a 35 magnification, the larvae of *Oesophagostomum columbianum* were collected, in some cases several hundreds of larvae being obtained from one sample. A small quantity of water was then added to the liquid in the watch glass, bringing the total volume up to about 5-10 c.c., which was then drawn up into a syringe and ejected into the mouth of the experimental sheep. Where the number of larvae in the mixture was sufficiently high, say several thousands, no further dosing of the sheep was undertaken (i.e. single infection or sudden intake), but where the sample supplied only a small quantity of larvae, then the injection was repeated at intervals of one to two days (i.e. progressive infection or gradual intake. See also section II.)

Ic. *Cultivation of the Larvae in vitro.*

In order to obtain daily information as to the progress of the infection, faeces were collected by means of a small bag placed under the tail, as illustrated in Fig. 2. The method worked quite satisfactorily, and no complications resulted from the contamination of the faeces by urine. The bag was emptied morning, afternoon and evening, and the three samples then amalgamated, a portion being placed into a fruit jar, broken up slightly, damped with water and the lid of the jar screwed down tightly. In the summer months, the jars were stored in a dark room at room temperature, whilst in the winter they were placed in an incubator running at about 25° C. With the object of preventing any abnormal fermentation deleterious to the growth of the larvae, pultaceous diarrhoeic faeces were mixed with autoclaved dry droppings. After storage the collection of the larvae of *Oesophagostomum columbianum* or of any other contaminating worm was then proceeded with in the manner previously described. As a general rule, during the development of the worm in the host, cultures made from faeces were found to be negative, but directly oviposition commenced in the host, the resulting larvae could be detected in the culture. Occasional tests were undertaken to ascertain whether the eggs passed out with the faeces of the host

were fertile, and also to see whether all the fertile eggs would develop to larvae. It was only on very rare occasions that normal development did not occur, and it can be accepted without hesitation that both these factors are so negligible that they do not affect the course of the curve showing the total daily output of eggs (see charts Nos. 1-4).

ID. *Counting of Larvae.*

The number of larvae that developed from the faeces was taken as a basis for calculating the number of eggs passed by the host. The total weight of faeces passed during 24 hours was found. Of this 5 grams were incubated in a tube for about a week. The contents of the tube were then shaken with 2 c.c. water and this was poured out into a graduated tube; a few c.c. of water were again added and the mixture allowed to stand for about two hours when this water was added to the first. The bulk of the water used for washing the culture was then made up to 20 c.c. and was well shaken in order to obtain a uniform suspension of larvae. 1 c.c. of the suspension of larvae was then poured into a glass evaporating dish, the base of which was divided into a number of small sections, and the level of the water was raised to about $\frac{1}{8}$th of an inch. The larvae quickly settled at the bottom of the dish and a count was then made of the number of larvae in each of ten sections. The average was struck and the figure then multiplied by the total number of squares on the base of the dish. Multiplication of the resulting total number of larvae by 20 then represented the number of eggs that were present in the original 5 gms. of incubated faeces, and the fifth of this figure represented the average number of eggs per gram of faeces. Multiplication of the average by the total gram weight of the faeces collected in 24 hours gave a figure representing the number of eggs passed out by the infected lamb during the 24 hours of observation. In this way it was possible to draw a curve representing the total daily output of eggs.

II.—THE RESULT OF THE ARTIFICIAL INFECTION.

Limiting Factors.—Gradual and sudden intake of larvae, age of host, seasonal conditions.

The following observations were undertaken with a view to ascertaining whether the infection with larvae is more effective when a comparatively small number of larvae are taken at intervals (gradual intake) or when the total infection is given at once (sudden intake). The object of repeated administrations of larvae was of course to endeavour to reproduce artificially a type of infection which approximates closely to the actual state of affairs on the veld.

The following table shows the results obtained amongst a number of lambs varying in age from a few days to more than twelve months, when infected with various numbers of larvae at different seasons of the year:—

TABLE I.

Lambs Artificially Infected with Oesophagostomum columbianum *Larvae.*

Date of Infection.	No. of Lamb.	Age of Lamb when Infected.	Number of Larvae Administered.	Interval between Date of Infection and First Appearance of Eggs in Faeces.	Death.	Season.
20. 7.22	7872	12 days..	2,000 in one drench	96 days...	Survived..	Winter.
5.12.23	7873	15 months	100 larvae every 3 days. Total, 2,000	41 days...	3 months.	Summer.
15. 2.24	7867	10 months	15,000 larvae in one drench	120 days..	5 months	Summer.
15. 2.24	7869	11 months	15,000 larvae. 1,000 larvae every day	93 days...	4 months	Summer.
17. 4.24	7863	16 months	2.000 larvae in one drench	60 days...	8 months	Winter.
17. 4.24	7864	16 months	2,000 larvae. 400 larvae every three days	52 days...	4 months	Winter.
17. 4.24	7865	15 months	1,000 larvae. 200 larvae every three days	52 days...	8 months	Winter.
25. 7.24	8483	10 months	15,000 larvae in one drench	60 days. *No larvae*	2 months (complication)	Winter.
25. 7.24	7853	11 months	15,000 larvae. 1,000 larvae every day	41 days...	2½ months	Winter.
28. 7.26	14512	4 months	1,600 larvae in one drench	38 days...	3½ months	Winter.

Conclusions.—From the table it can be seen that with a progressive or gradual infection the eggs appear in the faeces sooner and the infected sheep die sooner than is the case in the lambs treated with a sudden intake of larvae. Neither the age of the lamb nor the season of the year seemed to affect the comparison. It may be mentioned that lamb No. 7872 was killed at the age of 22 months when 16 adult Oesophagostomums (7 males and 9 females) were counted in the colon, and according to the experience of the writer, such an infection is a fair average and should not prove fatal.

III.—THE DEVELOPMENT OF THE INFECTION IN THE SHEEP.

IIIA. *The Period of Incubation.*

Two distinct periods can be recognised in the development of

the parasite in the host. First of all, a *period of incubation* represented by the larval stage of the worm, and secondly, a *period of maturity* during which oviposition commences. During the period of incubation, the larvae produce local intestinal reactions resulting in the formation of the larval cysts and of the parasitic nodules, a point to which reference was made in the article appearing in the Ninth and Tenth Report. During the period of maturity, the host becomes a carrier of infection by reason of the action of expelling eggs on to the pasture and thus creating a source of infection to susceptible animals. Careful observations were undertaken in connection with the period of maturity with the dual object of (1) estimating the degree of infection produced in the veld, and (2) ascertaining the duration of infection with regard to the longevity of the larvae in the intestinal nodule and of the adult worm in the lumen of the large intestine.

IIIb. *Period of Maturity.*

The Host as a Carrier of the Infection; Daily Oviposition of a Colony of Worms.

A newly born lamb was selected for this experiment and was subsequently infected every third day with 100 larvae of *Oesophagostomum columbianum*, until a total infection of 2,000 larvae were given (two months gradual intake), cultural examination of the faeces being undertaken daily. The interval elapsing from the date of infection until the first appearance of eggs in the faeces indicated "the period of incubation," and the daily count of larvae was utilised to plot out a curve showing the progress of the infection (see chart No. 1). Careful note was taken of the symptoms shown by the lamb during the progress of the infection, and on post mortem examination, a pure infection of *Oesophagostomum columbianum* was disclosed, there being 796 adult worms (280 males and 516 females) and 28 larvae in the fourth stage lying free in the lumen of the large intestine. At the post mortem examination 513 nodules were counted in the walls of the small intestine and 360 nodules in the walls of the large intestines, making a grand total of 873 nodules. The majority of these nodules were dry and calcareous whilst the remainder were yellowish-green and partly caseous or with calcified contents. No larvae were found in the calcareous and partly calcified nodules that were examined.

Observations on Chart No. 1 (Oesophagostomum columbianum *Infection*).

(1) The date of infection of the lamb corresponded with the season when lambs on South African veld are naturally infected, namely, early summer, and death occurred at the time when under natural conditions mortality from Oesophagostomiasis is noticed in South Africa, namely, in the early winter.

(2) Eggs were detected in the faeces 41 days after the commencement of artificial infection. It may be noted that this is one of the shortest periods that has so far been recorded by the writer in the life history of *Oesophagostomum columbianum*.

(3) From the 41st to the 83rd day of infection, the oviposition curve is comparatively low, due to the slow development resulting from progressive infection, but thereafter there is a sudden rise to a

distinctly higher daily average (two million daily), this sudden rise apparently being caused by the fact that all the adult worms had reached the stage of oviposition.

(4) Although the lamb died when the worms were in active oviposition, it can be concluded from the chart that the highest daily rate of oviposition was reached about 2½ months after infection was completed.

(5) During the period when the colony of worms was in oviposition, three distinct depressions can be noticed in the curve, namely from the 1st to 3rd February, from the 13th to 15th February, and from the 26th to 27th February. It is of interest to note that roughly corresponding with these periods, discharges of pultaceous, diarrhoeic, decomposed faeces were noticed. In the earlier report quoted, reference was made to the observation that in times of mucous diarrhoea numbers of larvae and adult worms may be passed out, and the tentative conclusion was drawn that the nature of the contents of the colon seems to be one of the factors controlling the presence of adult worms in infected sheep. A similar conclusion appears justified with regard to the presence of eggs in the faeces in the case of chart No. 1, although comparison with the other three charts shows that in each instance periods of very low oviposition precede periods of exceptionally high egg deposit.

(6) Generally speaking, oviposition steadily increased from a few thousands to several millions daily. In studying the curve day by day, numerous fluctuations above and below the steady increase can be seen and such variations illustrate the difficulty of placing too much reliance on the result of any form of treatment when determined solely by the number of eggs in the faeces. This point is noteworthy in view of the experience of other workers who have reported fairly constant results in series of egg counts with other species of worms when carried out over several days.

With the object of ascertaining whether the peculiar features of the curve shown were specific for *Oesophagostomum columbianum*, a further experiment was undertaken. Three lambs of about the same age and condition were selected and were injected artificially with 2,000 larvae respectively of *Haemonchus contortus*, *Trichostrongylus instabilis* and *Strongyloides papillosus*, and a curve was compiled showing the daily evacuation of eggs (see chart Nos. 2, 3 and 4). In the case of the lamb receiving *Trichostrongylus*, the count was maintained for twelve months.

Observations on charts Nos. 1 to 4. (Comparison between *Oesophagostomum columbianum*, *Haemonchus contortus*, *Trichostrongylus instabilis* and *Strongyloides papillosus*.)

In comparing the four curves, the following observations may be recorded:—

(1) The number of worm eggs passed out daily by an infected lamb is not constant.

(2) In each of the four species under consideration, the curve shows a gradual rise to a maximum ovipositon, which maximum is maintained for a few days, and although in the course of subsequent observations oviposition reaches a high level, the height of the first maximum is not attained.

(3) During the cold season—June, July, and August—the rate of oviposition is rather low, especially in the case of *Trichostrongylus*. With the return of the warm weather, there is a marked rise in the curve, although the height of the first maximum is not reached.

(4) Each of the four curves is marked by the similarity of sudden falls in the rate of oviposition, and in each case it would seem that there is a short period of low oviposition preceding a period of a corresponding high rate of oviposition.

IIIc. *Duration of the Infection.*

IIIc. *Longevity of the Larvae in the Intestinal Nodule of Lambs.*

Naturally infected lambs.—In the following table are recorded certain observations made on examination of fourteen lambs that were born at different Government Experimental Stations where *Oesophagostomum columbianum* infection is known to be severe, which died or were killed within eight months from the date of birth. The infections were contracted while grazing on the veld.

TABLE II.

Date of Observation.	D.O.B. No.	Age of Lamb at death.	Place of Observation.	Worm Infection at P.M. Examination.	Number of Nodules Present.
22. 9.21...	3821	3 months	B.	H.C., nil T., rather numerous O.C., adult, young, 20♂, 33♀	Caseous or calcareous nodules, 26 Infected, nil.
23. 9.21...	3820	4 months	B.	H.C., nil T., nil O.C., adult, 9♂ 3♀	Calcareous, 4. Infected, nil.
26. 9·21...	3819	2 months	B.	H.C., 5 adults T., some O.C., adult, young, 13♂, 9♀	Dry, small, 11. Infected, nil.
22.11.21...	3822	4 months	B.	H.C., numerous, adult T., some O.C., adult, numerous	Dry, calcareous, small, fairly numerous. Infected, nil.
10.12.21...	3824	4 months	B.	H.C., 8 adults T., a few O.C., 22♀, 13♂, adult, full-grown	Dry, calcareous, small, 15. Infected, nil.
17. 3.22...	N.N.	7 months	G.	H.C., nil T., adult, 19 O.C., adult, 12	Calcareous, 1. Infected, nil.
21.10.21...	4385	3 months	G.	H.C., adult, numerous T., some O.C., adult, 22	Calcareous, 4. Infected, nil.

TABLE II (*continued*).

Date of P.M. Examination.	D.O.B. No.	Age at Death.	Days of Infection.	Worm Infection at P.M. Examination.	Number of Nodules Present.
25.11.21...	N.N. (kid)	5 months	A.	H.C., adult, 1 O.C. nil	Calcareous, 2. Infected, nil.
13.10.21...	4384	3 months —	G.	H.C., adult numerous O.C., adult, 10	Calcareous, 1. Infected, nil.
20.10.21...	4383	3 months	G.	H.C., adult, numerous T., adult, 4 O.C., adult, 3	Calcareous, 1. Infected, nil.
19. 3.22...	N.N.	8 months	G.	H.C., adult, nil Trichuris, adult, 19 O.C., adult, 12	Calcareous, 1. Infected, nil.
20. 3.22...	N.N.	8 months	G.	H.C., adult, numerous O.C., adult, numerous	Calcareous, 5. Infected, nil.
15.12.21...	N.N.	6 months	B.	H.C., adult, 1 O.C., adult, 17	Calcareous, 10. Infected, nil.
27.12.21...	46	6 months	B.	H.C., adult, numerous O.C., adult, 77	Calcareous, 16. Infected, nil.

NOTE.—In column "Place of Observation," B = Bestersput, near Bloemfontein, O.F.S.; G = Grahamstown, Cape Province; A = Armoedsvlakte, Vryburg, Bechuanaland.

In column "Worm Infection at P.M. Exam.," H.C. = *Haemonchus contortus*; T = *Trichostrongylus*; O.C. = *Oesophagostomum columbianum*.

In colu nn "Number of Nodules Present," "infected" indicates number of nodules containing a larva of O.C.

Observations on Table.—In considering these observations, it must be assumed that the lambs were not infected before three to four weeks after birth as it would not be possible for them to feed on grass under the age of three weeks. Nodules, but without larvae of *Oesophagostomum columbianum*, were found in a lamb (see lamb 3819) one month after veld infection, but no infected nodules could be found amongst any of the 14 lambs under observation.

Artificially infected lambs.—In the following table, reference is given to the worm infection found on post mortem examination on lambs that were born and bred under laboratory conditions at Onderstepoort and at Vryburg, such lambs having been drenched artificially with mature larvae of the nodular worm:—

TABLE III.

Artificial Infection in Lambs.

Date of P.M. Examination.	D.O.B. No.	Age at Death.	Days of Infection.	Worm Infection at P.M. Examination.	Number of Nodules Present.
7.11.22...	40	5 months	65	H.C., Nil T., a few O.C., adult, very numerous	Calcareous, very numerous. Yellow caseous, some. Infected, nil.
3.11.22...	34	5 months	105	O.C., adult, numerous Larvae, 4th stage, nil	Calcareous, very numerous. Some green, cheesy nodules of which 25 per cent. contain a larva.
18.10.22...	33	4 months	99	O.C., adult, young, numerous Larvae, 4th stage, some	Large caseous (1 cm. in diameter), very numerous. Infected, 3 per cent.
8. 9.22...	32	2 months	50	O.C., adult, nil. Larvae, 4th stage, nil	Caseous, numerous. Infected, 80 per cent. Several open nodules showing a larva.
20.1.23...	50	57 days	22	O.C., adult, nil. Larvae in 4th stage, numerous	Closed and open caseous, numerous. Infected, nil. (Lamb died of septic peritonitis.)
17.1.23...	N.N.	29 days	19	O.C., adult, nil. Larvae, 4th stage, numerous	Closed and open caseous, numerous. Infected, nil. (Lamb died of septic peritonitis.)
13.11.22...	42	2½ months	40	O.C., adult, several Larvae in 4th stage, numerous	Closed caseous, numerous. Infected, 25 per cent.

TABLE III (*continued*).

Date of P.M. Examination.	D.O.B. No.	Age at Death.	Days of Infection.	Worm Infection at P.M. Examination.	Number of Nodules Present.
15.11.22...	44	2 months	56	O.C., adult, 21	Cheesy, closed, numerous. Infected, 25 per cent.
10. 8.21...	64	27 days	15	O.C., adult, nil Larvae, 4th stage, numerous	Caseous, closed, very few. Infected, 5 per cent. Open, ulcerated, numerous. Infected, 2 per cent. (Lamb died of septic peritonitis and necrotic colitis.)
12. 9.21...	N.N.	7 months	28	O.C., adult, 1 Larvae, 4th stage, 30	Caseous, closed, numerous. Infected, nil. Open, ulcerated, numerous. Infected, nil. (Lamb died of catarrhal necrotic colitis.)
9.3.23....	7873	19 months	75	O.C., adult, 794 Larvae, 4th stage, 28	Closed, 873. Calcified, great major. Green, cheesy, some. Infected, nil.
4. 5.21...	N.N.	2½ months	50	O.C., adult, 3	Closed, rare. Infected, 20 per cent.
12. 8.21...	N.N.	4½ months	106	O.C., adult, 100	Closed, calcareous, 6. Infected, nil.
31. 8.21...	N.N.	5 months	135	O.C., adult, 12	No nodules seen.
19. 6.22...	Kid	7 months	60	O.C. adult, numerous Larvae, 4th stage, numerous	Closed, caseous, cheesy, numerous. Infected, nil.
5. 9.25...	8532	12 months	90	—	Closed, caseous, cheesy, numerous. Infected, 30 per cent.

NOTE.—O.C. = *Oesophagostomum columbianum*; T = *Trichostrongylus*; "Days of Infection" = Number of days that elapsed between the date of artificial drenching with mature larvae and the date of death.

"Infected" indicates presence of a living larva in the nodule.

"Larvae, 4th stage" indicates larvae found free in lumen of large intestine.

Conclusions on longevity of the larvae in lambs.—In the lambs that were infected naturally as a result of grazing on the veld, nodules without larvae were found from the first month after infection. These nodules were calcareous and in the majority of lambs that were examined, there were more adult worms in the lumen of the intestine than there were nodules in the walls. This observation would seem to indicate that the larvae rapidly passed through the parasitic cyst to the intestinal lumen and that a large number of cysts healed up without leaving any trace to the naked eye. No larvae of the fourth stage were found in the lumen of the colon.

In the lambs drenched with mature larvae and subsequently kept in small camps, 25 per cent. to 80 per cent. of nodules were still infected within two months. In one lamb examined three months after infection, 30 per cent. of infected nodules were found, but it should be mentioned that the lamb was rather old, and it seems that in this case the exit of the larvae from the mucosa was delayed for a considerable time. No larvae were found in the intestinal nodules from the 16th week after infection.

Longevity of the Larvae in the Intestinal Nodule of Adult Sheep.

(a) *Naturally infected sheep.*—In Table No. 5 (Longevity of *Oesophagostomum columbianum* in artificially infected adult sheep) reference is given to several sheep which did not show larvae in the intestinal nodules twelve months after infection, but where all nodules were found to be calcareous That table contains practically all the information that is at present available to the writer on the point mentioned in the heading of this paragraph.

(b) *Artificially infected sheep.*—The following table is reprinted from the article on the "Preliminary Notes on the Life History of *Oesophagostomum columbianum*," which appeared in an earlier report. The infection was conveyed by the administration of mature larvae of *Oesophagostomum columbianum* through the mouth.

TABLE IV.

Artificial Infection of Adult Sheep.

Date of Observation.	D.O.B. No.	Days after Infection.	*Oes. Col.* Infection at the P.M.	Infected Nodules.
19.10.22	23	22	Larvae in 4th stage, fairly numerous	Caseous, green, closed. Infected, 50 per cent. Open. Infected, 10 per cent.
11. 6.23	403	30	Adult, full grown, 21 (old natural infection) Adult, very young, numerous Larvae in 4th stage, 7 (artificial infection)	Caseous, yellow. Infected, 50 per cent.
26. 5.21	1157	32	Larvae in 4th stage, numerous	Caseous, green, closed. Infected, 50 per cent. Open. Infected, 12 per cent.

TABLE IV (*continued*).

Date of Observation.	D.O.B. No.	Days after Infection.	*Oes. Col.* Infection at the P.M.	Infected Nodules.
16. 6.23	1505	35	Adult, full grown, 23 (old natural infection) Adult, very young, several Larvae, 4th stage, several (new artificial infection)	Caseous, yellow. Infected, 50 per cent.
23.12.22	56	68	Larvae in 4th stage, rather frequent	Cheesy, very frequent. Infected, 50 per cent. Open, several. Infected, nil. Calcareous, very frequent. Infected, nil.
14.12.22	4257	77	Adult, mature, 11 Larvae, 4th stage, 5	Cheesy, green, very numerous. Infected, 30 per cent. Open, numerous. Infected, 10 per cent.
3. 1.23	59	79	Adult, mature, 8 Larvae, 4th stage, nil	Cheesy, green, very numerous. Infected, 50 per cent.
21.12.22	4334	90	Adult, mature, 6 Larvae, 4th stage, nil	Cheesy, green, numerous. Infected, 40 per cent. Open, several. Infected, nil. Calcareous, numerous. Infected, nil.
27. 1.23	N.N.	105	Adult worms, nil Larvae, 4th stage, 4	Cheesy, green, numerous. Infected, 35 per cent. Calcareous, rather numerous. Infected, nil.
17. 3.23	11532	150	Adult worms, nil Larvae, 4th stage, nil	Cheesy, green, very numerous. Infected, 45 per cent. Calcareous, very numerous. Infected, nil. Open, several. Infected, nil.
20. 3.23	716	150	Adult worms, nil Larvae, 4th stage, nil	Cheesy, green, closed, very numerous. Infected, 25 per cent. Calcareous and partly calcareous, numerous. Infected, nil.
21. 3.23	110	150	Adult worms, 1 Larvae, 4th stage, nil	Cheesy, green, closed, very numerous. Infected, 65 per cent. Calcareous and partly calcareous without larvae, fairly frequent

TABLE IV (*continued*).

Date of Observation.	D.O.B. No.	Days after Infection.	*Oes. Col.* Infection at the P.M.	Infected Nodules.
3. 7.23	1306	52	Adult, full grown, 3 (old natural infection) Adult, young, 13 Larvae, 4th stage, a few (artificial infection)	Cheesy, green, numerous. Infected, 30 per cent. Open, several. Infected, 1 per cent. Calcareous, numerous. Infected, nil.
30. 7.23	64	75	Adult, mature, numerous Larvae, 4th stage, some	Cheesy, green, numerous. Infected, 30 per cent. Open, nil. Calcareous, frequent. Infected, nil.
30. 7.23	4270	105	Adult, mature, fairly numerous Larvae, 4th stage, nil	Cheesy, green, numerous. Infected, 50 per cent. Calcareous, several. Infected, nil. Calcareous, numerous. Infected, nil.
13.10.25	8365	180	Adult, full grown, 80 Larvae, 4th stage, nil	Cheesy, green, some. Infected, 20 per cent. Open, nil. Calcareous, numerous. Infected, nil.
15. 8.24	7864	120	Adult, full grown, 350	Practically all calcareous, 955 Green, cheesy, very few. Of these, 10 per cent. showed a larva.

Notes on Table IV.

(1) In sheep No. 110 when post mortemed five months after infection, about one-third of the nodules were found to contain living larvae whilst only one adult worm was present in the lumen of the large intestine.

(2) In sheep No. 8365 when post mortemed six months after infection, larvae were found in the intestinal nodules.

(3) In all cases where larvae were present, they were found to be living in the green, caseous or cheesy nodules. Neither larvae nor remnants of larvae were found in the calcareous nodules.

Conclusions on the longevity of larvae in adult sheep.—Larvae were passed out of the nodules into the lumen during the first two months after infection. Some of these larvae were then passed out to the open by the repeated excretion of diarrhoeic or soft faeces, and the rest reached the adult stage in the lumen of the intestine. In the third and fourth months it appears that a number of adult young worms were also forced out of the intestine whilst larvae were still passed out of the nodules, but only a small percentage reached the adult stage. Between the 100th and 156th days, open nodules were found, and it seemed that the larvae did not reach the adult stage, but were washed out of the intestine. Even after six months from the time of infection, larvae were still present in a number of nodules.

General Considerations on the Longevity of Larvae of Oesophagostomum columbianum.

From the tables it seems that in young lambs larvae find more favourable conditions for development than in adult sheep and that generally speaking, they migrate from the nodule to the lumen of the intestine within the first three to four months of their parasitic life. In the case of adult sheep, larvae were present in the intestinal nodules up to the sixth month after infection. Lambs and sheep infected under natural conditions do not appear to afford equally favourable conditions for the nodular worm as the artificially infected animals. It is worthy of note that amongst the hundreds of nodules that have been dissected by the writer, extending over a period of several years, neither dead larvae nor fragments of larvae have ever been detected. The larvae that were seen were invariably found in caseous or cheesy nodules but never in calcareous nodules. The question naturally arises whether the larvae leave the nodule by reason of incipient calcareous degeneration of the nodular contents, or whether this calcareous degeneration takes place only after the exit of the larvae. So far the evidence available seems to point to the latter possibility as caseous nodules without larvae are frequently found, indicating that the nodule is still caseous when the larva leaves.

IIIc. II. *Longevity of the adult worm in the lumen of the large intestine.*

Artificially infected sheep.—Ten adult merinos were artificially infected on the 4th April, 1925, with 2,000 larvae of the nodular worm (*gradual infection*). The merinos were placed in a camp at the Laboratory, were kept under close observation, and the progress of the infection as estimated by the calculation of the number of larvae present in cultured faeces is shown in the following table:—

(The result of infection was positive in only 4 of the 10 cases.)

TABLE V.

Longevity of Oesophagostomum columbianum *in Artificially Infected Adult Sheep.*

Date of Examination.	Sheep 6477.	Sheep 8448.	Sheep 6557.	Sheep 8365.
	Eggs.	Eggs.	Eggs.	Eggs.
12. 6.25	Very rare	0	0	0
21. 7.25	0	0	0	Several* (see note.)
13.10.25	0	0	0	
27. 5.26	A few	Several	Numerous	
27. 7.26	0	A few	A few	
31. 7.26	0	0	Frequent	
4. 8.26	0	Rare	Rare	
9. 9.26	0	A few	A few	
15.10.26	0	A few	0	
4.11.26	Several	0	Frequent	
14. 1.27	Frequent	Several	Frequent	
1. 2.27	0	A few	0	
3. 3.27	Rare	Several	0	
14. 4.27	Several	Several	0	

Notes on Table V.

(1) On post mortem examination of sheep No. 8365, 80 adult nodular worms were found, two larvae of the fourth stage, free in the lumen of the colon, and a number of parasitic nodules in the intestinal mucosa of which 20 per cent. contained a larva. In each case where the nodule contained a larva it was of the caseous variety.

(2) At the time of writing this article, the three infected sheep are still under observation, and judging by the small number of eggs present in the faeces and by the good condition of the sheep, it can be concluded that the number of individual worms present in the colon is comparatively small.

Artificially infected lamb.—Additional evidence concerning the longevity of *Oesophagostomum columbianum* in the host was obtained from casual observations carried out on a lamb No. 7872 in July, 1922, when the lamb was under one month old. This lamb received a " sudden " artificial infection of mature larvae and during the course of the infection, several attacks of diarrhoea were noticed accompanied by evacuation of a considerable number of adult nodular worms. The lamb was killed on the 26th March, 1924 (22 months old), when on post mortem examination 16 adult *Oesophagostomum columbianum* (7 males and 9 females) were found in the large intestine. The possibility of natural infection during the period of observation was excluded by the fact that the lamb had been kept in the special cage raised above the level of the ground as illustrated in Fig. 1.

Conclusions.—The experimental evidence justifies the conclusion that adult *Oesophagostomum columbianum* can live in the intestines of sheep for 20-21 months in the majority of cases, and in exceptional cases even longer.

Naturally infected sheep.—In table VII, particulars are given of the results of the field observations undertaken at the Veterinary Research Laboratory on the Experimental Farm at Armoedsvlakte, near Vryburg, in Bechuanaland.

TABLE No. VI.

Rainfall: Vryburg (Bechuanaland).

Date.	Rainfall.		
	Amount in Inches.	Rain began.	Rain ceased.
5. 1.22	·02	4.20 p.m.	5 p.m.
11. 1.22	·06	9 p.m.	9.30 p.m.
16. 1.22	·60	During night.	During night.
20. 1.22	·06	4 p.m.	4.30 p.m.
24. 1.22		2 p.m.	
25. 1.22	1·65		2 p.m.
26. 1.22	·07	4.20 p.m.	4.30 p.m.
2. 2.22	·65	6 p.m.	7 p.m.
8. 2.22	·07	7 p.m.	8.30 p.m.
17. 2.22	·09	6 p.m.	6.15 p.m.
22. 2.22	·05	5 p.m.	5.30 p.m.

TABLE VI (*continued*).

Date.	Rainfall.		
	Amount in inches.	Rain began.	Rain ceased.
3. 3.22	·35	3.45 p.m.	4.15 p.m.
9. 3.22	·15	6 p.m.	6.30 p.m.
24. 3.22	·20	3.30 p.m.	During night.
26. 3.22	·22	6.30 p.m.	8.30 p.m.
27. 3.22	·13	During night.	During night.
10. 5.22	·02	During night.	During night.
29. 5.22	·04	During night.	During night.
30. 5.22	·34	During night.	During night.
13. 6.22	·13	During night.	During night.
25. 8.22	·05	3.40 p.m.	3.50 p.m.
4.10.22	·02	During night.	During night.
22.10.22	·05	During night.	During night.
11.11.22	·10	During night.	6.45 a.m.
12.11.22	·71	3 p.m.	During night.
21.11.22	·34	7 a.m.	12 a.m.
22.11.22	·05	During night.	During night.
28.11.22	·07	5.10 p.m.	6 p.m.
11.12.22	·20	5.30 p.m.	During night.
12.12.22	·04	5.25 p.m.	5.40 p.m.
13.12.22	·72	2 p.m.	6 p.m.
16.12.22	·09	5 p.m.	During night.
17.12.22	·34	9.15 p.m.	During night.
18.12.22	·11	2.30 p.m.	3 p.m.
23.12.22	·08	7 p.m.	8 p.m.

The portion of the South African Continent in which the experiments were undertaken is comparatively dry, and judging from the rainfall figures for the year 1922, quoted on Table No. VI, the rainfall was insufficient to allow development of the *Oesophagostomum columbianum* eggs, voided out by the sheep, into the infective stage. Generally speaking, the possibility certainly does exist that mature larvae stored in the soil may fail to migrate on to the grass and hence not be available for ingestion by sheep, and in the case of the Vryburg experiment the observation made on post mortem examination of the three kids that died on the 19th and 25th November and 2nd December, 1921, respectively, gives useful information. No larvae were found in the intestinal nodules and the nodules were all calcareous, hence support is given to the contention that no infection took place during 1922. The majority of the sheep referred to in table No. VII had been previously running on the experimental farm at Bestersput, O.F.S., a farm that was known to be heavily infested with *Oesophagostomum columbianum*. The sheep were moved from the Orange Free State to Bechuanaland in November, 1921, and it is safe, therefore, to conclude that the infection in the sheep which died or were killed at Vryburg in 1922 was more than twelve months old.

Table No. VII.

Results of observations on sheep running under field conditions near Vrybury during the year 1922.

Date of Observation.	D.O.B. No. of Sheep.	Condition of Sheep.	Worm Infection at P.M. Examination.	Observations of Nodules.
12.10.21	11388	Adult, marasmatic, killed	H.C., 70 full grown T., numerous O.C., 398 full grown	Calcareous, very numerous. No larvae present.
12.12.21	1122	Fair, killed; faeces normal; fair number of eggs of H.C., O.C., and T.	H.C., 33 full grown O.C., 48 full grown	300 nodules. No larvae.
7.12.21	630	Adult, rather poor in condition, killed	H.C., nil O.C., 10 full grown	Dry, calcareous, numerous. No larvae.
7.12.21	2731	Adult, good condition, killed	H.C., nil T., nil O.C., several, adult	Large, caseous, numerous.
19.11.21 2.12.21	Two kids	Two and three months of age respectively	H.C., nil T., nil O.C., nil	Absent.
11.10.22	4331	Adult; killed *in extremis* for Marasmus	H.C., nil T., numerous O.C., numerous, full grown	Calcified, without larvae, very numerous.
11.10.22	4025	Adult; killed on account of Marasmus	H.C., frequent, full grown O.C., frequent, full grown	Calcified, without larvae, numerous.
12.10.22	2813	Adult; killed on account of Marasmus	H.C., nil O.C., fairly frequent	Calcareous, without larvae, numerous.
18.10.22	(?)	Adult; killed on account of Marasmus	H.C., frequent, full grown O.C., frequent, full grown	Calcareous, without larvae, numerous.
19.10.22	23	Adult, died; faeces normal. H.C. eggs, nil. O.C. and T. eggs, numerous	H.C., numerous T., numerous O.C., numerous, full grown	Calcified, without larvae.
25.10.22	438	Adult; killed on account of Marasmus	H.C., nil O.C., very numerous, full grown	Calcified, without larvae.
26.10.22	4242	Adult; killed on account of Marasmus	H.C., nil O.C., numerous, full grown.	Calcified, without larvae.

TABLE VII (*continued*).

Date of Observation.	D.O.B. No. of Sheep.	Condition of Sheep.	Worm Infection at P.M. Examination.	Observations of Nodules.
2.10.22	4155	Adult; marasmatic. Died as a result of gangrenous pneumonia	H.C., nil O.C., numerous, full grown	Calcareous, without larvae, frequent.
3.10.22	4225	Adult; killed *in extremis* of a Marasmus. Faeces normal. O.C. eggs, numerous	H.C., a few, full grown O.C., numerous, full grown	Calcareous, without larvae, not numerous.
9.10.22	3497	Adult; killed *in extremis* for Marasmus	H.C., nil O.C., very numerous	Calcareous, without larvae, frequent
8.11.22	2734	Adult; died of verminosis	H.C., nil O.C., numerous	Calcareous, without larvae, frequent.
20. 3.22	4315	Adult; marasmatic	H.C., adult, numerous O.C., adult, very numerous	Calcareous, very numerous. Infected, nil.
20. 3.22	4167	Adult; marasmatic	H.C., adult, frequent O.C., adult, frequent	Calcareous, very numerous. Infected, nil.

N.B.—H.C. = *Haemonchus contortus*; O.C. = *Oesophagostomum columbianum*; T. = *Trichostrongylus*.

Notes on Table VII.

(1) The sheep in the experiment were practically all adults.

(2) The absence of larvae in the nodules indicates that the infection was several months old and the dry winter weather which is distinctly unfavourable for larval infection of sheep indicates that the infection eventually found in the sheep was picked up previously to the winter.

(3) The presence of *Oesophagostomum columbianum* in sheep Nos. 1122, 630 and 2731 when post mortem examination was made in November and December, 1922, could not have been picked up during the dry winter months at Armoedsvlakte.

(4) Referring in some instances to the presence of eggs in the faeces, it may be mentioned that the specific identification was obtained by culturing the eggs and subsequently identifying the larvae.

Conclusions on Table VII.

(1) Fourteen sheep which had been running during the winter on a farm where the rainfall was practically nil, and which had obtained their water through a drinking trough, all showed an infection of *Oesophagostomum columbianum* (adult infective stage) and the majority of these sheep were severely infected.

(2) The *Haemonchus contortus* infection was noted in four out of fourteen sheep and only in one of these cases was the infection severe.

(3) From a comparison of the results of the cultural tests on sheep Nos. 1122, 23, and 4225, it would appear that the oviposition of *Haemonchus contortus* during the winter months is less constant than that of *Oesophagostomum columbianum*.

(4) It would also appear that in several of the sheep showing a pure infection of adult *Oesophagostomum columbianum*, their condition was reduced to a general atrophia terminating fatally.

(5) It is worthy of note that no larvae were found in the nodules.

(6) It would also appear that sheep kept under veld conditions are more susceptible to infection than sheep kept in a stable.

(7) Finally, it would seem from table VII that in adult sheep, although more resistant than lambs to *Oesophagostomum columbianum* infection, the infection may be followed by fatal results.

IV. Symptomatology of Oesophagostomiasis.

The pathology of Oesophagostomiasis comprises:—

(1) The local lesions in the intestinal mucosa produced by the larval stage of the parasites (traumatic nodular enterocolitis),

(2) The toxaemia resulting from peritonitis as a consequence of perforation of the intestinal wall, and

(3) The specific toxaemia caused by the larvae and adult parasites.

Artificial infections with pure strains of *Oesophagostomum columbianum* larvae were carried out systematically on both clean lambs and adult sheep, and the results that were obtained can be classified under three distinct groups:—

(1) Cases in which death occurred during the larval development of the worm, as a result of a septic condition of the intestine.

(2) Cases in which the worms developed to the full-grown stage and death occurred several months after infection as a result of a specific verminous intoxication.

(3) Cases in which the infection followed a mild course and resulted in the presence of a few adult worms in the lumen of the intestine, without causing death or appreciable illness of the host.

IVa.—*Traumatic and Septic Form of Oesophagostomiasis.*

As mentioned in the preliminary note in the 9th and 10th Report of the Director of Veterinary Education and Research, the form under discussion occurred in young lambs which were severely infected at an early stage. So far, 78 lambs, 2 days to 9 months old, have been artificially infected with larvae, the dosage varying from a total of 1,000 larvae administered daily in small numbers over several days to 70,000 larvae given in one drench. The penetration of the larvae through the intestinal mucosa gave rise to slight general symptoms, and a distinct rise of temperature lasting for several days was noticed in a number of cases of which a typical instance is illustrated in

chart No. 5. It is pointed out, however, that a similar febrile reaction was noticed in lambs infected with *Haemonchus contortus* and with *Trichostrongylus instabilis* as illustrated in charts Nos. 6 and 7, and it has to be considered that the rise of temperature was apparently due to the organic reaction of the host to the traumatic lesions of the digestive mucosa. On the fifth, sixth and seventh days after infection, diarrhoea was noticed, accompanied by shaking of tail, frequent micturition, thirst and grinding of the teeth. Invagination of the small intestine was also noticed at this stage with the typical symptoms which farmers call Reksiekte; the hind legs are stretched backwards, the fore legs are pushed forward, and the back is hollowed (see Fig. 11). From the 10th day mucous diarrhoea streaked with blood was noticed. Larvae of *Oesophagostomum* in the fourth stage were found to be numerous in the faeces in some cases. A complication of septic fibrinous and purulent peritonitis was also observed at this stage of the infection, apparently as a result of penetration of larvae from the intestinal parasitic cysts to the peritoneal cavity. Cases of death occurred on the 14th day after infection. In other cases, the lamb survived and the diarrhoea was followed by an attack of severe constipation with casual passage of dry, tar-like, blood-stained and mucoid faeces. In the majority of cases, attacks of constipation and diarrhoea alternated which ultimately proved fatal.

Pathological changes.—The morbid conditions of the intestine were found to vary from a nodular, ulcerative colitis to a general necrotic colitis. In severe cases, the nodular ulcerations produced by the passage of the larvae into the lumen showed ulcerative and necrotic complications, and these lesions were so close to each other as to reduce the mucosa propria to a thick, spongy, brown-grey layer, coated with blood-tinged mucus. Very few larvae were found in the lumen, as the majority had been passed out with the diarrhoeic discharges. In the above cases the traumatic destruction of the mucosa caused by the migration of the larvae resulted in a fatal necrotic colitis.

IVB.—*Toxic Form of Oesophagostomiasis.*

This stage in the infection was observed in lambs of one to two years old which resisted the nodular traumatic period of the disease and in which the worm grew to the adult stage. During the first and second month after infection, lambs suffering from the toxic form of the disease did not exhibit any very definite symptoms, and it was not until several months had passed that the acme was reached. In general, the appetite was good; there was occasional tympany of the rumen, whilst diarrhoea and constipation alternated similarly to the behaviour of subjects in the traumatic and septic form, although not so severe. The lambs were weak, frequently lay down and showed stiffness in gait. The conjunctiva and visible mucous membranes were not particularly pale. The typical "snow pale conjunctiva" of the sheep infected with wire worm was not noticed in the cases under discussion. Gradually the lambs lost flesh, and occasionally the abdomen was distended. Patches of the fleece could be pulled off from the skin. By this time, viz., about the third to fourth month of infection, a peculiar symptomatology was noticed in several cases.

The infected lambs showed a great tendency to lie down, and the hind quarters were affected by a pronounced muscular weakness. In the course of a few days, the lamb was no longer able to maintain the standing position, and was unable to rise or stand, even when lifted up. The cause appeared to be a paresis of the hind quarters, as in the repeated attempts to rise, the animal could use the forelegs, the neck and the head freely, but the hind legs could only be slightly moved. Both appetite and digestion were good. The act of mastication and all movements of the head were perfect, whilst the eyes functioned normally. The wool fell away and hung down in patches, giving the impression of a case of scab. If such a sheep were kept under shade and well fed, it would live for many days without being able to rise; in fact, one particular lamb that had been infected with 2,000 larvae showed the symptoms prescribed and survived for six weeks.

After daily observation of several cases of artificial infection in clean lambs, the writer has satisfied himself that the paresis of the hindquarters as described was not connected with the general weakness of the lamb. As a point of interest, one particular case of paresis in the front legs of a kid No. 9637 can be referred to. Apart from a gradual loss of condition, this animal did not show severe symptoms until the 155th day of infection when it was found walking on its knees, but still feeding well. This symptom persisted on the following day, and the kid died on the 157th day of infection, showing a pure but severe infection of adult *Oesophagostomum columbianum* in the large intestine. The nodules were very scarce and calcareous.

IVc.—*Anatomical Lesions.*

These can be summarised as general atrophy of the muscular system with large patches of complete alopecia, localized particularly on the neck, dorsal and lumbar regions. The conjunctiva was rather pale, the subcutaneous tissue was dry. The internal organs were rather pale; the liver, heart and spleen were particularly atrophic. The small and large intestines generally showed dry, small, calcareous, parasitic nodules without larvae. The nodules were small in number, in fact so small as to escape the notice of a casual observer. The parasitic worms were located in the large colon. The worms were in the adult stage, and varied greatly in numbers in lambs which were artificially infected with the same number of larvae. For instance, lamb No. 7873 showed 780 adult worms; in lamb No. 7864 there were 237 females and 113 males; in lamb No. 7865 there were 108 worms, whilst in lamb No. 7863 there were 389. In some cases the mucosa of the large colon was slightly thickened, whilst in others it was roughened. Diffuse patches of hyperaemia were also noted.

IVd.—*Oesophagostomiasis in Adult Sheep.*

Summary of conclusions.—In South Africa the adult merino sheep is more resistant to *Oesophagostomum* infection than the lamb. Artificial infection on stabled, adult sheep showed that the number of adult worms found was particularly small when compared with the number of larvae which were administered. The nodular infection was particularly mild both with regard to the number of nodules.

and to their local reaction. The infected sheep, although carriers of infection, did not show appreciable symptoms and put on weight when well fed. As it was mentioned in a previous note, the presence of the first eggs in the faeces of five infected adult sheep was noticed on the 52nd day after infection, and a fair number of eggs was found daily for weeks afterwards.

V.—Therapy of Oesophagostomiasis.

Various remedies such as Sodium arsenite, Copper sulphate, Cooper's Dip, etc., appear to affect *Oesophagostomum columbianum* infection to a certain extent and some individual worms are killed, but no remedy administered through the mouth seems to be uniformly satisfactory. The reason is that in the passage of the drugs through the alimentary system, they are partly absorbed or changed to such an extent that by the time they reach the site of *Oesophagostomum columbianum* infection, their anthelminthic effect is practically nil.

Va.—*Combined Anthelminthic and Purgative Action.*

With the object of carrying a remedy in the shortest possible time to the large intestine where the *Oesophagostomum columbianum* is located, and with the additional object of preventing or reducing intestinal absorption, it was decided to try a combination of purgatives with drugs of known anthelminthic effect. A comprehensive series of experiments was undertaken on a large number of sheep utilising the common purgatives such as Magnesium sulphate, Castor Oil, Croton Oil, Senna, Aloes, Cocoanut Oil, Cambogia, Elaterium, Podophyllin, etc.

Vb.—*Purgative Effect of Croton Oil in Sheep.*

The results of these experiments indicated that Croton Oil gave the best results and a special series of experiments was undertaken on sheep of different ages and in varying stages of condition. In the first experiments, the dose of Croton Oil utilized for adult merinos in good condition was 1 c.c., but no purgative effects were noticed. Doses of 2 to 4 c.c. were then tested, and with the former dose 82 per cent. of the infected sheep were purged, whilst with the 4 c.c. dose, the desired results were obtained in 100 per cent. The further observations that were undertaken at various times of the year on sheep in good and in poor condition with the object of ascertaining the maximal safe dose and the minimal efficient dose are summarised in the following table:—

TABLE No. VIII.

Purgative Effect of Croton Oil in Sheep.

Date of Dosing.	Purgative Used.	No. and Condition of Sheep.	Results. Days after Treatment. 1	2	3	4	5	6	7	
24.2.26	4 c.c. Croton Oil..	14, adult, strong	7D	14D	13D	13S	5S	2S		2
			7N		1N	1N	9N	12N	14N	X
24.2.26	4 c.c. Croton Oil..	10, adult, strong	9D	10D	9D	1D	1D			
	6 c.c. Cocoanut Oil					6S	3S	1S		
			1N		1N	3N	9N	9N	10N	
25.2.26	5 c.c. Croton Oil..	10, adult, strong	8D	10D	5D	5D				
					5S	2S	5S			
			2N			3N	5N	10N		
25.2.26	5 c.c. Croton Oil..	9, adult, poor	5D	8D	3D	3D	3D			2
	5 c.c. Cocoanut Oil				6S	2S	2S			X
			4N	1N		4N	4N	9N		
4.3.26	4 c.c. Croton Oil..	34, adult, poor	27D	34D	15D	9D				3
	6 c.c. Cocoanut Oil				9S	6S				
			7N			19N	36N			
14.4.26	4 c.c. Croton Oil..	22, adult, strong	12D	21D	14D	14D	6D	3D	2D	
	16 c.c. Crude Castor Oil				7S		4S	4S	2S	
			10N	1N	1N	8N	12N	15N	18N	22N
15.4.26	4 c.c. Croton Oil..	11, adult, strong	5D	9D	6D	4D	3D	1D		
	16 c.c. Crude Castor Oil				3S	2S		2S	1S	
			6N	2N	2N	5N	8N	8N		11N
15.4.26	3 c.c. Croton Oil..	4, adult, strong	1D	2D	1D	1D				
	17 c.c. Crude Castor Oil				2S					
			3N	2N	1N	3N	4N			
15.4.26	3 c.c. Croton Oil..	14, adult, poor	7D	14D	10D	8D	3D			1
	17 c.c. Crude Castor Oil				4S	2S	1S	2S	2S	X
			7N			4N	10N	12N	12N	12N

NOTE.—D. = Diarrhoea; S. = Soft faeces; N = Normal faeces; X = Dead.

Remarks on Table No. VIII.—All the 14 adult sheep dosed with 4 c.c. Croton Oil on the 24th February, 1926, purged, and five sheep ceased feeding that day, four were not feeding the following day, and two were not feeding on the fifth day of purging. Two sheep died, the one 15 and the other 18 days after dosing. All ten sheep used as controls on the 24th February, 1926, to ascertain whether the dilution of Croton Oil with some non-irritant oil would be safer, purged and although one sheep ceased to feed on the first day, one on the second and one on the fifth day, no mortality occurred. On the 25th February, 1926, before the final results of the above quoted experiments were known, 5 c.c. Croton Oil was used on ten sheep in good condition, and purging was produced in all instances; three

sheep ceased to feed on the day after dosing, but no mortality occurred. As a control to the 5 c.c. Croton Oil dose, nine adult sheep in poor condition were selected on the 25th February, 1926, and dosed with a mixture consisting of 5 c.c. Croton Oil plus 5 c.c. Cocoanut Oil. Four sheep ceased to feed the day after dosing and one on the fourth day. Purging was produced in all instances. Two sheep died, the one on the 8th day and the other on the 21st day after dosing. On the 4th March, 1926, the mixture of 4 c.c. Croton Oil plus 6 c.c. Cocoanut Oil was tested on 34 adult sheep in poor condition. Purging was noted but it did not persist any longer than in the other cases although the intestinal discharge was liquid, and blood and mucus was seen in some instances. Three sheep died out of the 34, the deaths occurring on the 4th, 17th and 20th days after dosing. On the 14th April, 1926, a mixture consisting of 4 c.c. Croton Oil and 16 c.c. Crude Castor Oil was tested on 22 adult sheep in good condition. Purging was observed over rather a long period, but the symptoms were not very severe and no mortality occurred. On the 15th April, a mixture consisting of 3 c.c. Croton Oil and 17 c.c. Crude Castor Oil was administered to four adult sheep in good condition, whilst as a control the same dose was used on a batch of fourteen sheep in poor condition. The sheep in good condition resisted the dose well although purging was noticed for a comparatively long period of four to six days. The batch of sheep in distinctly low condition, which were anaemic and were below the condition usually observed in sheep running on the veld, resisted the 3 c.c. Croton Oil dose fairly well, although one animal in a very marasmatic condition and anaemic, died, the lesions of diffuse acute hyperaemia being found on post-mortem examination, extending from the abomasum to the large intestine.

Provisional conclusions.—A mixture consisting of 4 c.c. Croton Oil plus 6 c.c. Crude Castor Oil is apparently the maximal safe dose for good conditioned sheep, but it is probably too strong for general purgative purposes. Such a mixture may not cause purging in all cases, whilst in a certain percentage, the effects may be prolonged for too lengthy a period. A mixture of 4 c.c. Croton Oil plus 16 c.c. Crude Castor Oil is milder than the maximal safe dose, although nevertheless, it produces purging over a lengthy period. Croton Oil in the dose of 4 c.c. is too severe for verminous sheep in poor condition and the maximal safe dose for this class of animal appears to be 3 c.c. Croton Oil plus 17 c.c. Crude Castor Oil. Owing to the fluctuating rate at which the purgative flows through the digestive tract of ruminants, the dose which in the great majority of sheep proves to be effective and safe will nevertheless produce some fatal cases and in some instances, no effects at all. Generally speaking, the addition of a comparatively large amount of a mild oil such as Linseed or Crude Castor, decreases the severity but lengthens the period of purging.

Vc.—*Croton Oil plus Anthelminthic in Adult Sheep.*

Several experiments were undertaken on sheep of different condition, using Croton Oil as the purgative plus an inorganic anthelminthic (Sodium Arsenite plus Copper Sulphate) in contrast to an organic anthelminthic (Filmaronic Acid). In the following experiments the inorganic anthelminthic was administered in the form of the Government Wire Worm Remedy, which consists of

four parts of finely ground Copper Sulphate and one part of 80 per cent. of Arsenite of Soda. The results of the trial dosings are summarized in the following table:—

TABLE No. IX.

Purgative Effect of Croton Oil plus Anthelminthic in Adult Sheep.

Date of Dosing.	Purgative Anthelminthic Used.	No., Age, and Condition of Sheep.	Days after Treatment.						
			1	2	3	4	5	6	7
8.2.27	5-notch spoon, G.W.W.R.	5, adult,	2D	4D	5D	1D			
	3 c.c. Croton Oil.....	strong	2S	1S		3S	1S		
	17 cc.. Crude Castor Oil		1N			1N	4N	5N	
13.3.26	5-notch spoon, G.W.W.R.	3, adult,		3D	2D	2D	1D	1D	
	3 c.c. Croton Oil.....	fair							
	7 c.c. Crude Castor Oil..		3N		1N	1N	2N	2N	
8.2.27	4-notch spoon, G.W.W.R.	10, adult,	6D	7D	9D	1D			
	2 c.c. Croton Oil.....	weak	1S	2S		2S	2S		
	18 c.c. Crude Castor Oil		3N	1X		6N	7N	8N	
13.3.26	4 gms. Filmaronic Acid	3, adult,		2D	2D	2D			
	3 c.c. Croton Oil.....	fair	1X						
	3 c.c. Castor Oil.....		2N				2N		
17.2.27	4-notch spoon, G.W.W.R.	2, two-	1D	2D	2D	1D			
	2 c.c. Croton Oil.....	tooth,				1S	1S	1S	
	18 c.c. Crude Castor Oil	strong	1N				1N	1N	2N
17.2.26	4-notch spoon, G.W.W.R.	10, two-	2D	10D	10D	5D			
	1 c.c. Croton Oil.....	tooth,	2S			2S	4S	3S	
	19 c.c. Castor Oil.....	poor	6N			3N	6N	7N	10N
17.2.26	3-notch spoon, G.W.W.R.	4, ten-	3D	4D	4D	2D			
	1 c.c. Croton Oil.....	month					2S	1S	
	19 c.c. Castor Oil.....	lambs	1N			2N	2N	3N	4N

NOTES.—G.W.W.R. A mixture of copper sulphate and sodium arsenite in the proportion of 4 to 1, which is largely used in South Africa against Wire Worm in sheep.

One 5 notch spoon dose contains mgr. 500 copper sulphate plus 125 mgr. sodium arsenite.

One 4 notch spoon dose contains mgr. 400 copper sulphate plus 100 mgr. sodium arsenite.

One 3 notch spoon dose contains mgr. 300 copper sulphate plus 75 mgr. sodium arsenite.

Filmaronic acid is the active principle of male fern.

D. = Diarrhoea. S. = Soft faeces. N. = Normal faeces. X = Dead.

Remarks on Table No. IX.—The results of the dosing on the 8th February using 3 c.c. Croton Oil plus Anthelminthic in adult sheep in good condition showed that the purgative effect was general and not too drastic, whilst with the adult sheep in poor condition used as controls, but dosed with a smaller bulk of Castor Oil, one showed severe diarrhoea in which were detected streaks of blood. Of the ten adult sheep in poor condition dosed on the 8th February with 2 c.c. Croton Oil plus anthelminthic, again the results were generally not too drastic, although one animal died from acute gastro-enteritis. Using the dose of 3 c.c. Croton Oil plus anthelminthic on the 13th March on three adult sheep in fair condition was followed by one death 24 hours later. On the 17th February, using 2 c.c. dose of Croton Oil plus anthelminthic, the result was again not too drastic. As a control, and on the same date, ten two-tooth sheep in fair condition were given 1 c.c. of Croton Oil in the mixture and the result was again satisfactory and not too drastic. With the four lambs that were dosed on the 17th February, the result was not too drastic.

Provisional Conclusions.

(1) It would seem that a dose of 3 c.c. Croton Oil was successfully maintained by adult sheep in good condition when mixed with a mineral anthelminthic.

(2) Croton Oil in the dose of 3 c.c. and of 2 c.c. when mixed with a mineral anthelminthic was too drastic for sheep in fair and in weak condition respectively.

(3) 3 c.c. Croton Oil when mixed with an organic anthelminthic was too drastic for adult sheep in fair condition.

(4) 1 c.c. to 2 c.c. Croton Oil mixed with an inorganic anthelminthic (Wire Worm Remedy) did not seriously affect two-tooth sheep in fair condition.

(5) Croton Oil in the dose of 1 c.c. plus the inorganic anthelminthic (300 mgms. Copper Sulphate plus 25 mgms. Sodium Arsenite) mixed with 19 c.c. of Castor Oil proved satisfactory and the purgative effect was not too drastic on lambs of ten months old.

(6) It would seem that Croton Oil is more effective and is safer when mixed with a fair amount of Castor Oil.

Vd. *Croton Oil plus Anthelminthic in Small Lambs.*

Some observations were also undertaken on the effect of the administration of Croton Oil plus Filmaronic Acid suspended in Crude Castor Oil administered to small lambs and the results can be summarised in the following table:—

TABLE X.

Purgative Effect of Croton Oil plus Anthelminthic in Small Lambs.

Date of Dosing.	Purgative and Anthelminthic Used.	No., Age, and Condition of Lambs.	Result. Days after Treatment. 1	2	3	4	5	6	
23.3.26	Filmaronic Acid, ·5 grm. Croton Oil, 10 drops.... Castor Oil, 8 c.c.....	1 lamb, 14 kgr., emaciated	N	N	D	N			
27.4.26	Filmaronic Acid, 1·5 grm. Croton Oil, 25 drops.... Castor Oil, 15 c.c.....	Same lamb, 14 kgr., emaciated	N	D	D	D	D	D	N
13.4.26	Filmaronic Acid, 1 grm. Croton Oil, 10–15–20 drops Castor Oil, 20 c.c.....	8, lambs, 11–20 kgr., strong	2D 6N	1D 7N	8N				
19.5.26	Filmaronic Acid, 1 grm. Croton Oil, 20–25 drops Castor Oil, 20 c.c.....	Same 8 lambs, 15–25 kgr., strong	5D 3N	3D 5N	1D 7N	8N			

D. = Diarrhoea. S. = Soft faeces. N. = Normal faeces.

Remarks on Table No. X.—In explanation of the above table, it might be mentioned that in the experiment of the 23rd March, the purgative effect of 10 drops Croton Oil plus anthelminthic on the six month old lamb was of a very mild nature and did not commence until a lengthy interval had elapsed. On the 27th April the same lamb was utilised when it was evident that the dose of 25 drops of Croton Oil was rather drastic. On the 13th April, the eight lambs that were dosed with varying quantities of Croton Oil, according to body weight, did not show any ill effects. When the same lambs were dosed just over a month later, one failed to purge whilst the others showed purging from one to two days.

Conclusions.—It was provisionally concluded that a dose of 20 to 25 drops of Croton Oil suspended in Castor Oil was a safe average dose for lambs of 10 to 25 kgms. body weight, provided the condition of the lambs was not too low.

General Observations on the Effect of Purgatives and Anthelminthics on Oesophagostomum columbianum.—The results obtained from mixing Croton Oil with Anthelminthics, such as Laboratory Wire Worm Remedy and Filmaronic Acid have shown that the remedies are far more effective against nodular worms when administered in combination with a purgative. It was, however, noted that the destructive effect on the worms depends to a very large extent on the presence and degree of purging rather than on the anthelmintic used, and also that the real vermifuge action was the result of purging. Further observations on this point are in hand and it is hoped that a more detailed report will be published later. Incidentally, it may be mentioned that for the purpose of obtaining comprehensive evidence on the effect of anthelminthics on any kinds of worms, it is essential to utilise a comparatively large number of individual hosts, and further, that in the case of *Oesophagostomum columbianum*, the artificial infection required for the experiments is not always constant, a small percentage of the sheep resisting infection with the result that the degree of infection varies very considerably in any particular batch.

Ve.—*The Anthelminthic by Enema.*

Following on the lines suggested by Brumpt, who administered Thymol by means of an enema against *Oesophagostomum columbianum*, a number of infected sheep were dosed per rectum with various drugs and the results can be summarized as follows:—

(1) Lamb No. 8480, about six months old, was infected with 1,500 mixed larvae of *Haemonchus contortus* and *Oesophagostomum columbianum* on the 25th August, 1924. Two months later the lamb was treated for Haemonchosis and on the 3rd March, 1925, the animal was found to be severely infected with pure *Oesophagostomum columbianum*. The lamb was in an extremely marasmatic condition, unable to stand, and showing lesions of decubitus (*vide* Fig. 12). A mixture consisting of 50 c.c. of an acacia gum solution plus 1 gm. of Thymol suspended in the solution was then administered by enema, and the following day, after a good number of adult nodular worms had been excreted, a distinct improvement was noticed. On the 7th March the enema treatment was repeated, when several other adult *Oesophagostomum columbianum* were passed out. The animal continued to improve and on the 10th March was able to stand. On the 17th March, the enema treatment was repeated for the third time,

but no worms were excreted and the cultural test on the faces proved negative for nodular worm larvae. Figure No. 13 shows the condition of the lamb three months later.

(2) On the 5th April, 1926, eleven adult sheep previously artificially infected with a mixed Strongylosis (Wire Worms, Nodular Worms, Trichostrongyles and *Strongyloides papillosus*) were examined and showed marked symptoms of verminosis. Cultural test of the faeces revealed the presence of *Oesophagostomum columbianum* in large numbers. In all sheep the colon contents were found to be dry. Ten days later, the sheep were given an enema containing Thymol and the results are shown in Table No. XI:—

TABLE No. XI.

Enema Treatment on Artificially Infected Sheep.

D.O.B. Reference Letter of Sheep.	Time of the Cultural Test.	Condition of Colony.	Specific Identification of Larvae and Percentages.	
			Oes. Col.	Other Species.
			%	%
L	Before treatment.....	Strong colony.....	37	63
	After treatment.....	Strong colony.....	10	90
K	Before treatment.....	Fair colony.....	60	40
	After treatment.....	Strong colony.....	0	100
K1	Before treatment.....	Strong colony.....	77	23
	After treatment.....	Strong colony.....	0	100
H	Before treatment.....	Fair colony.....	63	37
	After treatment.....	Strong colony.....	2	98
G	Before treatment.....	Strong colony.....	84	16
	After treatment.....	Strong colony.....	0	100
E	Before treatment.....	Larvae on walls.....	29	71
	After treatment.....	Larvae on walls.....	20	80
I	Before treatment.....	Fair colony.....	80	20
	After treatment.....	Fair colony.....	0	100
A	Before treatment.....	Strong colony.....	33	67
	After treatment.....	Fair colony.....	0	100
F	Before treatment.....	Strong colony.....	49	51
	After treatment.....	Strong colony.....	0	100
L	Before treatment.....	Strong colony.....	67	33
	After treatment.....	Strong colony.....	0	100
K2	Before treatment.....	Strong colony.....	49	51
	After treatment.....	Strong colony.....	2	98

Notes on Table No. XI.—Sheep L, K, H and G each received 1 gm. of finely pulverised Thymol suspended in 1,000 c.c. of acacia gum solution. Some difficulty was experienced in administering the enema owing to the very dry condition of the colon, but out of the five treated sheep, two were completely freed from nodular worm. The remaining sheep, K1, E, I, A, F, L and K2 received 1 gm. of finely pulverized Thymol suspended in 1,500 c.c. of acacia gum solution, but administering this enema with such a large amount of liquid proved to be very laborious. Out of the six treated sheep, five were completely freed from nodular worms.

(3) On the 22nd April, 1926, a number of sheep known to be severely infected with the usual Strongylidae, were subjected to enema treatment, the solution containing an anthelminthic. Examination of the faeces of each sheep was undertaken by cultural

method immediately before treatment and again 15 days later. The details of the composition of the enema and the results obtained are summarized in the following table:—

TABLE No. XII.

Enema Treatment Plus Anthelminthic on Artificially Infected Sheep.

D.O.B.	Time of the Cultural Test.	Condition of Colony.	Specific Identification and Percentage of Larvae.	
			Oes. Col.	Other Species.
			%	%
	Thymol: 2 grm. in 1,000 c.c. soapy water—			
N	Before treatment....	Fair colony.....	63	37
	After treatment.....	Strong colony.....	0	100
D	Before treatment....	Larvae on walls.....	36	44
	After treatment.....	Strong colony.....	0	100
C	Before treatment....	Fair colony.....	32	48
	After treatment.....	Strong colony.....	0	100
	Thymol: 1 grm. to each 10 lb. body weight in 1,000 c.c. soapy water—			
D	Before treatment....	Strong colony.....	45	55
	After treatment.....	Larvae on walls.....	1	99
M	Before treatment....	Strong colony.....	15	85
	After treatment.....	Strong colony.....	24	76
Cl	Before treatment....	Strong colony.....	15	85
	After treatment.....	Strong colony.....	0	100
	Government Wire-Worm Remedy: grm. 1·250 in 1,000 c.c. liquid—			
8519	Before treatment....	Larvae on walls.....	48	52
	After treatment.....	Strong colony.....	0	100
8571	Before treatment....	Strong colony.....	14	86
	After treatment.....	Strong colony.....	2	98
	Government Wire-Worm Remedy: grm 1·250 in 100 c.c. liquid, followed by 900 c.c. of water—			
G	Before treatment....	Fair colony.....	56	44
	After treatment.....	Fair colony.....	0	100
	Government Wire-Worm Remedy: grm. 0·625 in 1,000 c.c. of liquid—			
8547	Before treatment....	Strong colony.....	26	74
	After treatment.....	Fair colony.....	3	97
6093	Before treatment....	Strong colony.....	10	90
	After treatment.....	Strong colony.....	0	100
	Government Wire-Worm Remedy: grm. 0·625 in 100 c.c. liquid, followed by 900 c.c. water—			
B	Before treatment....	Strong colony.....	86	14
	After treatment.....	Strong colony.....	93	7
	Arsenious Oxide: grm. 1 Copper Sulphate grm. 1 in 1,000 c.c. liquid—			
9968	Before treatment....	Strong colony.....	80	20
	After treatment.....	Larvae on walls.....	0	100

Notes on Table No. XII.—It is worthy of note that the colony of larvae was in many cases found to be stronger after treatment than before, but this fact is explained by the presence of other species of round worms which are located in the small intestine, particularly *Strongyloides papillosus*, which in common with the others may greatly increase in numbers within a comparatively short time. Judging from the results of the two experiments with Thymol, it would seem that the effect on the worms is not in direct proportion to the actual dose of Thymol administered. Sheep M, which received Thymol and sheep B, which received Laboratory Wire Worm Remedy, strenuously resisted the application of the enema and from the results it would seem that the enema did not proceed along the full length of the colon.

Conclusion.—All the anthelminthics used in the enema treatment had a distinct vermicidal effect on the nodular worm, but it seems to be quite clear that a thorough washing of the lumen of the colon is essential for satisfactory results. Should the colon be in the normal or dry condition, the probability is that the enema will not prove successful.

VI.—Preventive Licks against Oesophagostomiasis and other Verminosis in Sheep.

A series of observations were also carried out to ascertain whether "Licks" containing anthelminthic principles would be harmful to mature and immature *Oesophagostomum columbianum*.

In order to obtain some reliable results, it was found necessary to consider various conditions in relation to the ingredients of the lick and to the natural habits of the animal.

VIa.—*Ingredients of the Lick.*

As a result of observations carried out for more than two years, it was ascertained that certain ingredients could be classified as *nourishing* (bone meal), some as *tonics* and *digestives* (calcium chloride, potassium carbonate), and some as *anthelminthics* (Arsenic, Copper sulphate, Nicotine).

It was observed that bone meal helped the sheep to withstand the debilitating action of the worms, but did not affect the parasites, that Loogas (plant ash containing a high percentage of Potassium salts) did stimulate the digestion and the appetite of the infected animals, but was ineffective against the parasites.

VIb.—*Craving Capacity of Sheep.*

Generally speaking, it is necessary to accustom sheep to partake of licks and it requires at least 15 days before the majority of sheep take tobacco leaf eagerly, whilst about 5 per cent. refuse entirely or are quite erratic in adopting the practice. It was also found that sheep kept in the stable are more easily accustomed than those sent to pasture, and that with the appearance of young luxuriant grass on the veld, infected sheep take less tobacco leaf, bone meal, etc., than when the grass is dry.

It was also observed that the amount of tobacco leaf or bone meal ingested by an infected sheep varies from day to day. Expressed in chart form the intake of lick shows that within a period

of about 10-15 days there is first of all a progressive rise reaching a maximum, followed about half way through by a progressive decrease reaching the minimum again and succeeded by repetitions of similar curves.

VIc.—*Selection of Anthelminthics.*

Guided by the experience gained with previous experimental work on the chemotherapy of Haemonchosis in sheep, it was decided to test drugs which pass through the alimentary canal practically unchanged, viz., the arsenical series and Copper sulphate, and also active principles which are intimately enclosed in vegetable cells and are presumably carried along the digestive tube, viz., Chenopodium plant and tobacco leaf. Loogas was also included in the series, as it was reported by farmers as very beneficial when licked by sheep infected with Strongylidae.

VId.—*Composition of Licks.*

For the experimental test, each lick included an anthelminthic plus common salt to induce sheep to take the lick, and in some cases bone meal was added in order to experiment on the combined anthelminthic and nourishing action of the lick.

VIe.—*Infection of the Experimental Sheep.*

It was thought that while the observations were being carried out on *Oesophagostomum columbianum*, the sheep could simultaneously be infected with *Haemonchus contortus*, *Trichostrongylus* and *Strongyloides papillosus*, as the conclusions would be of a greater practical importance.

VIf.—*First Experiment.*

The first experiment was started in May, 1924, and was practically closed in September, 1924. The number of sheep used for the experiment consisted of 120 lambs and 16 adult sheep, which were obtained from the veld and were divided as follows:—

Crushed tobacco leaf moistened with saturated sodium chloride solution: 52 animals.

Bone meal 1,000 parts plus sodium chloride 100 parts (in weight): 14 animals.

Bone meal 1,500 parts plus sodium arsenite 2.5 parts plus sodium chloride 100 parts (in weight): 14 animals.

Bone meal 1,000 parts plus sodium arsenite 1 part plus copper sulphate 4 parts plus sodium chloride 100 parts (in weight)*: 14 lambs.

Loogas 1,000 parts plus 250 parts Sodium chloride (in weight): 14 animals.

Chenopodium green leaves: 14 animals.

Control: 14 animals.

Excluding the " Tobacco " lot which ran in a bare camp, each of the other batches was kept in a separate stable box. The sheep

* It might be repeated that the Sodium arsenite and Copper sulphate in the proportion of 1 and 4 respectively, as mentioned in 4th lick, are the two components of the " Government Wire Worm Remedy," as called and used with success in South Africa against Wire Worm in sheep (see the Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918). For the sake of brevity, the lick will be referred to in the following pages as G.W.W.R.

were gradually accustomed to their respective licks until the end of June when the percentage in each batch that were freely licking was as follows:—

Tobacco leaf: 94·3 per cent.
Bone-meal: 100 per cent.
Bone-meal, plus Sodium arsenite: 78·6 per cent.
Fresh Chenopodium leaves: 100 per cent.
Loogas, 85·8 per cent.

The average daily consumption of lick was:—

Tobacco leaf, plus salt: from 3 to 11 grams.
Bone-meal plus salt: from 10 to 17 grams.
Bone-meal plus sodium arsenite plus salt: from 5 to 10 grams.
Bone-meal plus G.W.W.R.: from 4 to 10 grams.
Fresh Chenopodium leaves: from 3 to 64 grams.
Loogas: from 8 to 12 grams.

Judging by the daily average intake of Bone meal plus G.W.W.R., each sheep in that batch received an amount of G.W.W.R. corresponding to five times the dose prescribed by this Laboratory for monthly dosing.

The sheep received a " gradual artificial infection " of mixed larvae consisting of approximately:—

Oesphagostomum columbianum	20 per cent.
Haemonchus contortus	40 per cent.
Trichostrongylus	23 per cent.
Strongyloides papillosus	7 per cent.

The total number of larvae administered to each sheep was about 15,000 extending over the period from the end of June to the beginning of August, 1924

The larvae were administered in a watery suspension, either spread on grass or syringed into the mouth in 10 c.c. of water or infected directly in the rumen through the flank, and it was found out that there was no difference in the results obtained from the three methods The advantage of the two latter methods was that each sheep received the same number of larvae.

The body weight of each sheep was recorded every month, when a cultural test of the faeces was also made for the estimation of the strength of the colony of larvae and the percentage of each species. The monthly records of weight and infection were tabulated for each batch, and a monthly serial test was thus obtained.

In Tables XIII and XIV are given the results of the cultural tests, with the body weights of the various sheep in the experiment.