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SIGNIFICANCE OF INHIBITED DEVELOPMENT IN THE EPIDEMIOLOGY OF *CHABERTIA OVINA* AND *OESOPHAGOSTOMUM VENULOSUM* INFECTIONS IN SHEEP

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ABSTRACT

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Worm counts from ewes, lambs and tracer lambs during a study on the epidemiology of gastro-intestinal nematode infections in sheep at Utrecht State University between 1974 and 1977 substantiated the following descriptions of the epidemiology of *Chabertia ovina* and *Oesophagostomum venulosum* infections.

Development of inhibited larvae and recently acquired infection from pasture leads to a spring rise in the numbers of adult worms and developing stages in lactating ewes and some barren yearling sheep. However, in barren ewes and some barren yearling sheep very few adult worms develop.

The low residue of overwintered larvae on pasture results in infection of the lambs in spring and early summer. These larvae develop without inhibition. Numbers of infective larvae on pasture remain low until the autumn. This autumn increase leads to substantial worm burdens in lambs and ewes, but a high proportion of these worm burdens, especially in ewes, consists of inhibited larvae. A decrease in pasture larval contamination occurs during winter and the propensity of the larvae for inhibited development diminishes. The lambs lose most of their worms during winter.

Experimental infection of worm free sheep shows that it is possible to condition infective larvae, especially of *O. venulosum*, for inhibition of the development, by storage of the larvae for 5 weeks at 15 or 4° C.

INTRODUCTION

Inhibition of development is a well known phenomenon in many gastro-intestinal nematodes of ruminants (Michel, 1974). In sheep, inhibited larvae can continue their development before parturition and during lactation and contribute to the spring rise (Connan, 1968; Blitz and Gibbs, 1972). Especially in *Haemonchus contortus* (Connan, 1968, 1971; Blitz and Gibbs, 1972; Eysker and Hendrikx, 1977) and to a lesser extent *Ostertagia* spp. (Connan, 1968; Reid and Armour, 1972; Ayalew and Gibbs, 1973; Eysker and Hendrikx,

1977) and *Trichostrongylus* spp. (Eysker, 1978), inhibited development is of epidemiologic significance.

Connan (1974) described inhibited development of *Chabertia* in young sheep. He observed a development of the inhibited larvae in the beginning of the year associated with diarrhoea in some of the sheep.

In a long term study on the epidemiology of gastro-intestinal nematodes of sheep at Utrecht University the following observations were made on *Chabertia ovina* and *Oesophagostomum venulosum*.

MATERIAL AND METHODS

During 1974—1977, experimental sheep grazed three adjacent pastures 1, 1 and 0.5 ha each. These pastures were almost permanently grazed by sheep and could be considered highly infested with infective larvae of strongylids when the weather conditions were suitable for the development and survival of the free living stages of these worms.

The sheep were of the Texel or Frisian breed or Texel/Frisian crossbreds. Male lambs were castrated before the breeding season. In winter the sheep were given supplementary hay and concentrate. Before the start of the experiment the ewes used in 1975—1976 (groups E1—5, 7 and 9) had an almost life-long history of grazing sheep pastures which were highly infested with strongylid larvae. They were housed in April 1975 and treated with 100 mg/kg Thiabendazole on 4 June 1975.

The ewes used in 1976—1977 (groups E6 and 8) grazed pastures highly infested with sheep strongylids from the time of their purchase in September 1975. The lambs remained worm-free until they were turned out to pasture. The history of the lambs and the ewes is summarized in Table I.

A series of tracer lambs, reared under worm-free conditions, grazed the pastures in 1975—1976 and 1976—1977, usually for a period of 2—4 weeks. They were then rehoused under worm-free conditions and killed after 14—17 days.

Housed animals were fed hay and concentrate and had free access to water.

In addition, a series of experimental infection trials was carried out. The experimental design of these is summarized in Table II.

At necropsy the caecum was removed, opened, washed and discarded. The ingesta and washings were bulked in a container, water was added to make a total volume of 3 or 4 l. The same procedures were followed for the colon.

After stirring with a vibromixer for 15 min, three 30 or 40 ml aliquots and one of 210 or 240 ml, depending on the total volume of the container, were obtained. Formalin was added to make a dilution of 5%.

The samples were washed on a screen with a mesh diameter of 100 μm and stained by adding an alcoholic solution of iodine 5%. The ingesta was then decolorized using sodium thiosulphate solution. The worms, remaining brown for

TABLE I

History of the groups of lambs (L1—11) and ewes (E1—9)

Group	Number of animals	Breed*, sex of sheep	Age at start of experiment	Date put out to pasture	Date of housing	Housing period before necropsy (days)
Lambs slaughtered in the grazing season 1974—1975						
L1	4	F, ♀	6 months	29/9/1974	6/11/1974	26—30
L2	4	T, ♀	6 months	29/9/1974	6/11/1974	104—107
Lambs slaughtered in the grazing season 1975—1976						
L3	5	F, w	2 weeks	26/4/1975	25/8/1975	16—21
L4	5	F, w	2 months	4/6/1975	3/11/1975	14—16
L5	5	T, ♀	6 months	25/8/1975	3/11/1975	16—18
L6	4	T, ♀	6 months	25/8/1975	29/12/1975	14—16
L7	4	F, w**	2 months	4/6/1975	8/3/1976	14—16
Lambs slaughtered in the grazing season 1976—1977						
L8	5	T, w, ♀	2 weeks	26/4/1976	21/9/1976	0
L9	5	T, w	2 weeks	26/4/1976	27/10/1976	0
L10	5	T, w, ♀	2 weeks	26/4/1976	8/12/1976	12—13
L11	4	T, w, ♀	2 weeks	26/4/1976	7/3/1977	0
Barren ewes slaughtered in autumn, winter and spring						
E1	7	F, ♀	3—6 years	4/6/1975	25/8/1975	14—22
E2	8	F, ♀	3—6 years	4/6/1975	3/11/1975	14—18
E3	7	T, ♀	3—6 years	25/8/1975	3/11/1975	18—23
E4	7	T, ♀	3—6 years	25/8/1975	29/12/1975	15—18
E5	2	F, ♀	3—6 years	4/6/1975	8/3/1976	17—23
E6	9	T, ♀	2 years	26/4/1976	3/3/1977	0—18
Lambing ewes slaughtered within 3 days of parturition						
E7	5	F, ♀	3—6 years	4/6/1975	8/3/1976	16—36
E8	5	T, ♀	2 years	26/4/1976	3/3/1977	9—12
Lambing ewes slaughtered 6 weeks after parturition						
E9	3	F, ♀	3—6 years	4/6/1975	26/2/1976	54—62

*T = Texel; F = Frisian.
**w = wether.

some time, could be collected and counted quite easily. It was not possible to differentiate fourth stage larvae of *Oesophagostomum venulosum* and *Chabertia ovina* until they were close to the fourth moult, not even after comparison of fourth stage larvae of both species obtained from sheep infected experimentally with only one of the species.

TABLE II

Design of artificial infection experiments

Experiment number	Group	Breed*, sex of sheep	Number of lambs	Age (months)	Month of infection	Dose per sheep***	Exposure conditions	Days between infection and necropsy
1	a	T ♂	5	3	June 1975	1000 *C.* + 50 *O.*	—	25—27
	b	T ♂	5	7	October 1975	440****	—	32—33
2	c	T w**	2	13	February 1978	3400 *C.* +400 *O.*	—	35
	d	T w	3	14	March 1978	3400 *C.* +400 *O.*	35 days; 4°C	35—36
	e	T w	3	14	March 1978	3400 *C.* +400 *O.*	35 days; 15°C	35—36

*Texel.
**w = wether.
*** About 20% of the infective *Chabertia/Oesophagostomum* larvae could not be differentiated with complete certainty.
****Infective larvae not differentiated.
C. = *Chabertia*; *O.* = *Oesophagostomum*.

RESULTS

Naturally infected sheep

The numbers of worms in the different groups of ewes and lambs are summarized in Table III.

Ewes. Ewes slaughtered in March/April 1976 and March 1977 within 3 days of parturition (groups E7 and 8) harboured predominantly developing stages (late L_4 and L_5) of *C. ovina* and *O. venulosum*, whereas in barren ewes slaughtered in the same periods (groups E5 and 6) and in ewes killed in November 1975 (groups E2 and 3) and January 1976 (group E4), the bulk of the populations consisted of early L_4. In lactating ewes killed 6 weeks after parturition in

TABLE III

Chabertia ovina and *Oesophagostomum venulosum* populations in naturally infected ewes and lambs. The history of the animals is summarized in Table I

Group	Mean number per sheep of						
	Adult *C. ovina*	Adult *O. venulosum*	Late L_4/L_5 *C. ovina*	L_5 *O. venulosum*	Late L_4 of both species	Early L_4 of both species	Total
Lambs							
L1	30	5	22	0	5	355	417
L2	53	14	2	25	15	17	137
L3	180	30	0	0	0	0	210
L4	100	60	30	0	50	460	700
L5	90	80	20	0	70	430	690
L6	100	156	87	40	87	700	1170
L7	0	0	12	38	0	50	100
L8	22	24	2	4	4	12	68
L9	42	14	4	6	8	44	118
L10	50	6	12	0	10	600	678
L11	42	60	15	13	20	30	180
Ewes							
E1	50	7	21	0	7	29	114
E2	13	6	6	0	0	200	225
E3	14	0	0	0	0	50	64
E4	0	0	0	0	0	100	100
E5	0	0	0	0	0	175	175
E6	5	0	0	0	0	30	35
E7	40	0	90	10	110	0	250
E8	0	0	10	0	52	8	70
E9	583	50	0	0	0	0	633

April 1976 (group E9) adult worms were found. The ewes slaughtered in September 1975 (group E1) harboured adult worms, developing stages and early L_4.

Lambs. In group L1, which grazed from September to November 1974 the majority of the large intestinal strongyle populations found at slaughter in December 1975, consisted of early L_4. In group L2, which was killed 2.5 months later, these early L_4 had almost disappeared and somewhat more adult and developing worms were present.

During 1975 and 1976 an increase of the worm burden was observed until December (groups L3—6, L8—10), followed by a decrease until March (groups L7, 11). In November—December 1975 worm burdens in lambs (L4—6) were higher than those in ewes (E2—4), but by March this was no longer true (L7, E5 and 7). In autumn 1976 (L8—10) the number of worms tended to be somewhat lower than in 1975 (L4—6).

In both 1975 and 1976, the proportion of early fourth stage larvae was greater in lambs housed in November—December (groups L4, 5, 6, 10) than in those housed earlier (groups L3, 8, 9). By the spring, proportionately more adult and developing stages were found (groups L7, 11) than in November—December (L4, 5, 6, 10), although in March 1977, 110 out of 120 worms were still early L_4 in one of the animals.

Tracer lambs (Table IV). In 1975—1976 substantial worm burdens were found in tracer lambs grazing from the middle of September to the beginning of March, but especially in T5, 6 and 8, grazing in November, December and February. Between the middle of September (T3) and the end of December (T6) the majority and sometimes all of the worms found were early L_4.

In the beginning of September (T2) and in the winter and early spring (T7, 8 and 9) large proportions of the worm populations consisted of developing stages or adult worms. Fifth stage *C. ovina* were seen only in T7, 8 and 9 and fifth stage *O. venulosum* in T7 and 8 and also in T2 and 3.

In 1976—1977 much lower worm burdens were seen. Between June 1976 and March 1977 only five out of 11 tracer lambs contained worms.

Particularly remarkable in view of the results for T6 which grazed in December 1975, was the finding of only fifth stage *C. ovina* and *O. venulosum* in T19, which grazed during December 1976.

Experimental infections (Table V)

In groups a, b and c, which were infected with freshly cultured larvae, a small proportion of the worms present 25—36 days after infection were early fourth stage larvae, but in animals infected with stored larvae (groups d and e) a much higher proportion of the worms were early fourth stage larvae especially when the larvae were stored for 5 weeks at 4°C (group d).

The recognisable stages of *C. ovina* were all L_5 or late L_4, but of *O. venulosum* most were adult worms. In groups d and e, the number of L_5 and late L_4 of

TABLE IV

Chabertia ovina and *Oesophagostomum venulosum* populations in tracer lambs

Group number	Period on pasture	Mean number per sheep of						
		Adult *C. ovina*	Adult *O. venulosum*	Late L_4/L_5 *C. ovina*	L_5 *O. venulosum*	Late L_4 of both species	Early L_4 of both species	Total
T1*	11/ 8/1975—25/ 8/1975	0	0	0	0	0	0	0
T2*	25/ 8/1975—15/ 9/1975	0	25	0	25	50	50	150
T3*	15/ 9/1975—13/10/1975	0	100	0	0	0	325	425
T4	13/10/1975— 3/11/1975	0	0	0	0	0	450	450
T5	3/11/1975— 1/12/1975	0	0	0	0	0	3500	3500
T6	1/12/1975—29/12/1975	0	0	0	0	50	7000	7050
T7	29/12/1975—26/ 1/1976	0	100	150	50	0	350	650
T8	26/ 1/1976—17/ 2/1976	0	750	600	0	200	250	1800
T9	17/ 2/1976— 8/ 3/1976	0	0	100	0	200	100	400
T10	29/ 6/1976—13/ 7/1976	0	0	0	0	0	0	0
T11	13/ 7/1976—27/ 7/1976	0	0	0	0	0	0	0
T12	27/ 7/1976—10/ 8/1976	0	0	0	0	0	0	0
T13	11/ 8/1976—25/ 8/1976	0	0	0	0	0	0	0
T14	24/ 8/1976— 7/ 9/1976	0	0	0	0	0	50	50
T15	7/ 9/1976—21/ 9/1976	0	0	0	0	0	0	0
T16	21/ 9/1976— 5/10/1976	0	0	0	0	0	0	0
T17	5/10/1976—19/10/1976	0	0	100	0	0	100	200
T18	19/10/1976— 2/11/1976	0	0	0	0	0	50	50
T19	23/11/1976—21/12/1976	0	150	200	0	0	0	350
T20	6/ 1/1977—16/ 3/1977	0	20	0	0	0	0	20

Single lambs used except where * indicates groups of two.

TABLE V

Numbers of *Chabertia ovina* and *Oesophagostomum venulosum* in experimentally infected sheep. For experimental design see Table II

Group letter	Exposure conditions	Mean number per sheep of						
		Adult *C. ovina*	Adult *O. venulosum*	L_5/late L_4 *C. ovina*	L_5 *O. venulosum*	Late L_4 of both species	Early L_4 of both species	Total
a	—	0	14	172	0	32	26	244
b	—	0	84	110	4	16	22	236
c	—	0	175	90	0	50	95	410
d	35 days; 4°C	0	10	273	3	27	493	807
e	35 days; 15°C	0	27	483	0	113	437	1060

C. ovina was considerably higher, but of adult *O. venulosum* considerably lower than in group c. Late fourth stage larvae, which were not yet distinguishable, were present in all groups, but only in low numbers in group d. The establishment of infection was low except in group b; it was lower in group c, than in groups d and e.

DISCUSSION

The housing period of most naturally infected sheep was too short to enable recently acquired *C. ovina* larvae to develop beyond the fourth stage (Herd, 1971). This was well illustrated by the finding of late fourth stage larvae which could not be differentiated and of late fourth and early fifth stage *C. ovina*, in experimentally infected animals 4—5 weeks after infection. These late fourth stage larvae were probably mainly *C. ovina* because, in contrast to that species, most fifth stage *O. venulosum* were then fully mature adults.

The majority of the early fourth stage larvae must be considered inhibited in their development for the following reasons.

(1) The early fourth stage larvae were very uniform in size and mostly they were accompanied by adults and late developmental stages of *C. ovina* and *O. venulosum*, so the characteristic bimodal size distribution seen in partly inhibited worm populations was observed.

(2) In group L2, which had been housed for 3.5 months, early fourth stage larvae were still present. This group had been housed from 6 November onwards in an open roofed pen, which was cleaned thoroughly every week. Though during a short period in December the average temperature was high enough to facilitate development of infective larvae, reinfection could be excluded in the conditions prevailing in the pen.

Inhibited development occurs in both species. The finding of developing stages of *O. venulosum* in group L2 and the presence of more fifth stage *O.*

venulosum in group L2 than in group L1, is sufficient proof of the occurrence of inhibited development in this species. Evidence for inhibited development of *C. ovina* is less clear, but strong indications for the occurrence of inhibition in this species are given below.

(1) The finding of even low numbers of developing stages of *C. ovina* in lambs which had been housed for 3.5 months (group L2).

(2) The finding of developing *C. ovina* in lambs (L11) and ewes (E8) in March 1977, while in a tracer lamb grazing during January and February 1977 (T20) no *C. ovina* was found.

(3) Some of the early fourth stage larvae found in tracer lambs in autumn 1975 (T3 to 6) were almost certainly *C. ovina*, because the tracer results in the first months of 1976 (T7—9) show the presence of infective larvae of *C. ovina* on pasture during these months. As the temperature then was too low for development of infective larvae of *C. ovina*, these infective larvae must have been present already in the autumn.

Inhibited development has been described recently in *C. ovina* (Connan, 1974), and the results of Goldberg (1951, 1952) suggested an occurrence of inhibited development in *O. venulosum*.

The worm counts of the tracer lambs in 1975—1976 indicate that infective larvae acquired in autumn have a higher tendency to inhibit their development than larvae acquired earlier or later, because the proportion of early fourth stage larvae was higher in the tracer lambs T4—6 than in T2 and 3 and T7—9. This implies that the preparasitic stages are conditioned to inhibit their development by environmental conditions prevailing in autumn and subsequently are deconditioned in winter. Such a seasonal pattern of inhibited development is well known for several trichostrongylids of cattle and sheep (Reid and Armour, 1972; McKenna, 1973; Armour and Bruce, 1974; Michel, 1974; Eysker, 1979).

Consequently the developing stages found in ewes and yearling sheep in spring could be the result of resumption of the development of inhibited larvae or of larvae recently acquired.

The proportion of early fourth stage larvae is higher, and that of developing stages lower, in barren ewes (E2—6) than in lambs (L4—7) killed between November and March. This indicates that in addition to autumnal environmental conditions, host resistance is a factor in inhibition.

A comparison of data from ewes killed within 3 days (E7 and 8) and 6 weeks (E9) after parturition with those from barren ewes killed at the same periods (E5 and 6), suggests that host resistance may be a factor in the resumption of development.

Some additional information on the aetiology of inhibited development was obtained from the experimentally infected animals. When infective larvae were given to sheep after a culture period of 7 days at 25°C only a small portion of these larvae appeared to be conditioned to inhibit development. Storage of larvae at 15 or 4°C for 5 weeks was quite effective in conditioning the larvae to inhibit development, especially in *O. venulosum*, because in experiment 2 the absolute number of adult *O. venulosum* was much lower when the

larvae were stored for 5 weeks, than when the larvae were not stored, though the total number of worms was much higher. This effect of storage is well known in trichostrongylids (McKenna, 1973; Armour and Bruce, 1974; Michel, 1974) and generally is thought to be associated with ageing of infective larvae in late summer and autumn.

From the present results the following epidemiological pattern can be constructed.

(1) Lactating ewes and some barren yearling sheep contaminate the pasture with eggs of both species in spring; this spring rise is caused by resumption of the development of inhibited larvae and by recently acquired larvae.

(2) The low residue of overwintered larvae on pasture results in infection of the lambs in spring and early summer; these larvae develop without inhibition.

(3) Comparison of the numbers of trichostrongyles (Eysker, 1979) and strongyles in the tracer lambs in 1975 and 1976 shows that strongyle eggs are translated later than trichostrongyle eggs into infective larvae, leading to an 'autumn increase' of pasture larval contamination. Pasture larval counts in some earlier less dry years also showed this 'autumn increase' of the strongyles (W.M.L. Hendrikx, unpublished data, 1972—1974).

(4) The autumn increase leads to considerable worm burdens in lambs and to a lesser extent in older animals. Large proportions of these worm burdens, especially in older sheep, consist of inhibited larvae.

(5) During winter a decrease in the pasture larval contamination occurs and the propensity of the larvae for inhibited development is diminished; the lambs lose most of their worms during winter.

Connan (1974) observed chabertiosis in yearling sheep in the first months of the year caused by resumption of the development of inhibited larvae. Because overwhelming numbers of abomasal and small intestinal trichostrongyles accompanied the strongyle burdens in the lambs in the present experiments, clinical signs of chabertiosis were masked. Nevertheless, the adult *C. ovina* burdens found in most lambs in autumn and winter were, compared with those found by Herd (1971), sufficient to cause appreciable pathogenic effects and indeed substantial damage of the colon mucosa has been observed in many lambs post mortem. Consequently, *C. ovina* has to be considered as a pathogen in The Netherlands contributing to gastro-intestinal helminthiasis especially in autumn and winter.

Although according to Goldberg (1952) *O. venulosum* can also be of some clinical importance, the numbers found of this species were such that it can be considered as quite harmless under the circumstances at Utrecht State University.

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