The Effects of Age and Previous Infection on the Development of Gastrointestinal Parasitism in Cattle

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SUMMARY

An age resistance in cattle to establishment of infection with Cooperia oncophora was not demonstrated. Cattle exposed to a heavy infection for the first time at approximately 15 months of age were as susceptible to establishment of infection as 3 to 4 month old calves, but stunting of worms and inhibition of ovulation did occur in the older animals, possibly due to a rapid development of resistance as a result of sensitization by a previous extremely light infection.

An age resistance in cattle to infection with Nematordirus helvetianus was not clearly demonstrated. At necropsy, 8 of 9 calves and 2 of 6 yearlings exposed to pasture infections for the first time did harbour Nematodirus worm burdens, while yearlings which were heavily infected previously were completely free of this species.

Under the conditions of this investigation, age and acquired resistance to Ostertagia ostertagi were not demonstrated, since previously non-exposed calves and yearlings and previously infected yearlings had comparable worm burdens.

This study demonstrated the adverse effect that heavy parasitism has on the development of susceptible animals. Animals which had little or no exposure to parasitism were found to be much more susceptible to the effects of parasites than were resistant animals.

INTRODUCTION

During 1962 and 1963, studies on gastrointestinal parasitism in calves grazing on marshland pasture indicated that a rapid build-up of resistance and a shedding of parasites had occurred for Cooperia oncophora and Nematodirus helvetianus but not for Ostertagia ostertagi (11). Studies were initiated in 1964 to investigate these phenomena further and to determine what effect previous infection would have on the development of gastrointestinal parasitism in yearlings. The results of these studies, which extend over a period of 55 weeks, are contained in this report.

MATERIALS AND METHODS

A diagram illustrating the experimental design is given in Fig. 1. Nineteen parasite-free Grade Holstein calves, obtained in mid-March, 1964, at 1 to 3 days of age, were divided by random selection into groups A and B, of 12 and 7 animals, respectively, and reared indoors as previously described (11). On June 30, 1964, group A calves were placed in a three-acre paddock in which heavily parasitized calves had grazed in 1963, while the group B calves were placed on a two-acre pasture which had remained free of cattle and sheep for two grazing seasons. During the ensuing grazing season, five group A calves and one group B calf either died or were killed and all were examined for parasites. The surviving animals of each group remained on pasture until stabled in separate pens on October 28, 1964. During the stabling period, all animals were fed approximately 1 lb./day of a commercial dry cow ration and hay *ad libitum*. The pens were cleaned throughly each week and bedded with clean shavings. On January 20, 1965, one animal from group A was killed and its parasites counted. On June 23, 1965, (51 weeks after the initial exposure of group A) the six remaining animals in each of groups A and B, now yearlings, were put out to graze on the same three-acre infected pasture used by

Fig. 1. Diagram illustrating the experimental design.

The observations made on groups A and B calves will be considered during three periods: (1) 1964 pasture period, (2) overwinter stabling period, and (3) 1965 pasture period.

1964 PASTURE PERIOD

Clinical signs of parasitism were observed in the group A calves during the second and third weeks after they entered the infected pasture in June. The calves became listless, lethargic, unthrifty, and rough-coated in appearance. A marked diarrhoea, which developed in several animals toward the end of the second week, was present in all animals by the end of the third week. Loss in weight (Fig. 2) was associated with the diarrhoea. Concurrent with the diarrhoea and weight loss was the presence of helminth eggs in the
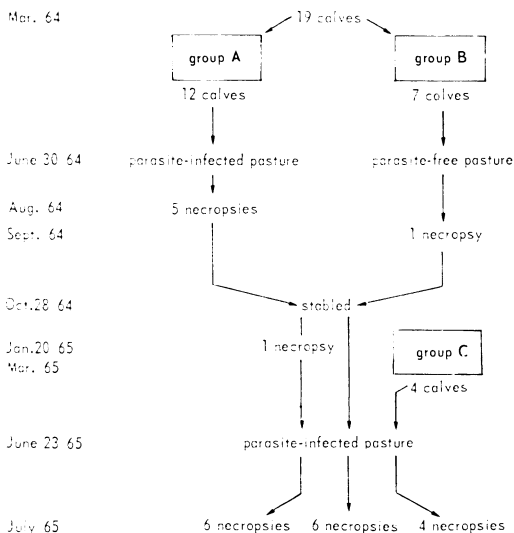
group A the previous grazing season. At the same time, four parasite-free calves (group C) obtained in mid-March, 1965, were put out to graze with the yearlings. All 16 animals were slaughtered and examined for parasites between 28 and 32 days later.

Blood samples were taken weekly from groups A and B animals from the day of purchase until the termination of the experiment. The serological studies on these samples are reported elsewhere (9,10). Calf weighings and fecal examinations were performed weekly from the beginning of the grazing period, June 30, 1964, to the termination of the studies in July, 1965. During the last week of the experiment in 1965, daily fecal egg counts were made. All fecal examinations were performed either by a simple flotation method, using super-saturated sodium nitrate as the flotation solution, or by the McMaster technique. The flotation method was used until an animal began to pass eggs, at which time the McMaster quantitative method was employed.

At necropsy, the gastrointestinal tracts from all animals were labelled and fixed in 10% formalin. Parasitological examinations were carried out by passing the washigs from the opened gastrointestinal tracts through a stack of four sieves (Fisher's U.S. Standard Sieve Series Nos. 10, 20, 40 and 65). The parasites were examined, identified and counted using Swales' method (12).
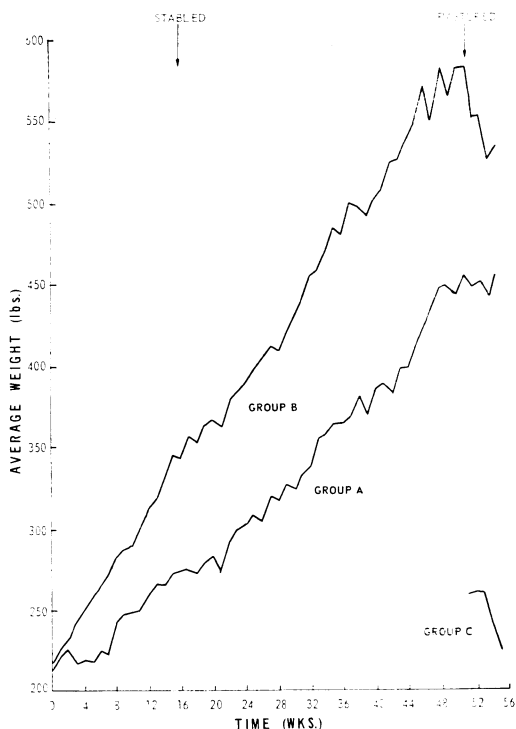

Fig. 2. Average weekly weight gains or losses of both parasitized and parasite-free calves from June 30, 1964, to July 20, 1965. Group A — exposed to heavily infected pasture both as calves and as yearlings. Group B — exposed to heavily infected pasture only as yearlings. Group C — exposed to heavily infected pasture only as calves. (Averages are for those animals remaining at each weighing.)

feces of all group A calves by the twenty-first day on the pasture. The worm egg output rose rapidly, reaching a mean of 1650 eggs per gram (e.p.g.) by the ninth week (Fig. 3). Maximum egg counts for individual calves ranged from 700 to 3,350 e.p.g., indicating that large infections undoubtedly had been established in all animals. It was during this period of high worm egg output that the calves were the most unthrifty. Calves A-1 and A-2 died on days 39 and 51, respectively, while calf A-3 was in a moribund condition when killed on day 51. The calves remaining after the period of weight loss and the marked unthriftiness, slowly began to gain weight and improve in appearance and condition. Accompanying this physical improvement was a dramatic reduction in worm egg output, which dropped to a very low level by the sixteenth week.

Clinical signs of parasitism did not develop in the group B calves grazing on the parasite-free pasture. As indicated in Fig. 2, fairly regular weight gains were recorded and, at the time of stabling, group B calves weighed an average of 83 lbs. more than the surviving group A calves. During the latter part of the grazing season, an occasional worm egg was recovered from the feces of several group B calves. This was attributed to contamination of the pasture by feces, probably carried in on footwear and weighing equipment.

OVERWINTER STABLING PERIOD

As indicated by average weight gains, the group A animals did not do as well as those in group B during the early winter months, even though both groups were on identical rations. In late winter, the group A animals began to make gains comparable to those in group B. By the end of the stabling period in June, 1965, group A animals were in as good condition but, as a result of earlier stunting, weighed, on the average, 128 lbs. less than group B.

Shortly after being stabled at the sixteenth week following initial exposure to the infected pasture, the group A calves had a second, but smaller, rise in worm egg output, which again coincided with a period of poor weight gains. The counts rose to an average peak of approximately 300 e.p.g. in 4 to 5 weeks' time and then gradually dropped to a very low level (50 e.p.g. or less) during the next 7 to 8 weeks. Worm egg counts remained very low ex-
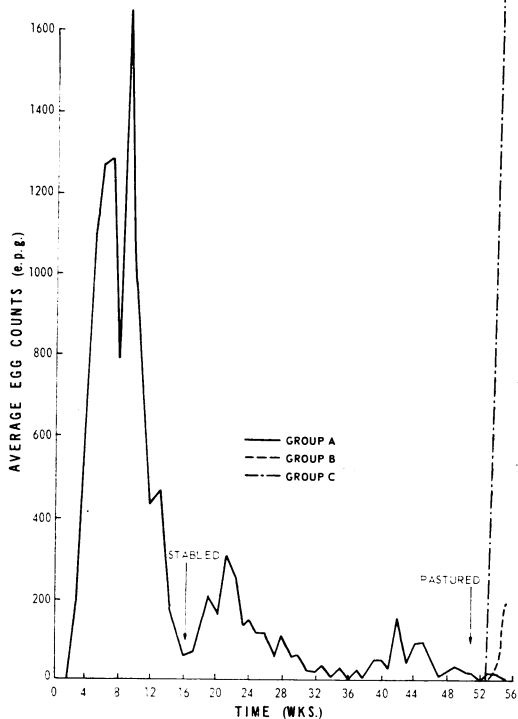

Fig. 3. Average weekly worm egg counts (e.p.g.) of parasitized calves from June 30, 1964, to July 20, 1965. Group A — exposed to heavily infected pasture both as calves and as yearlings. Group B — exposed to heavily infected pasture only as yearlings. Group C — exposed to heavily infected pasture only as calves. (Averages are for those animals remaining at each sampling.)

cept for a third small rise during several weeks in early spring (Fig. 3).

Throughout the stabling period, fecal samples from the group B animals were negative, except for the sporadic finding of an occasional worm egg.

1965 PASTURE PERIOD

During this grazing period, the group A yearlings gained an average of 2 lbs. per animal even though small losses in weight were recorded during the first 3 weeks on the infected pasture. On the other hand, the group B yearlings and group C calves lost an average of 49 lbs. and 37 lbs. per head, respectively, during the same period. The group B yearlings and group C calves developed a severe diarrhoea and became gaunt and thin. The group C calves had maximum egg counts ranging from 1,750

to 4,050 e.p.g., associated with this diarrhoea, while only animals B-2 and B-6 of group B passed worm eggs regularly, with a peak of 950 e.p.g. recorded for yearling B-6. The rise in mean egg counts for the group B yearlings shown in Fig. 3 is due largely to the findings in animals B-2 and B-6. Group A yearlings did not exhibit any increase in worm egg output during this grazing period.

PARASITES RECOVERED

The most common worm eggs recovered during this experiment were gastrointestinal helminth eggs (*Cooperia* and *Ostertagia*, as indicated by recovery of adults at necropsy), although small numbers of *Nematodirus* and *Trichuris* eggs were also present from time to time. *Nematodirus* eggs were never obtained from animals A-3, A-4, A-5 and A-6 of group A, and animals B-1, B-2 and B-7 of group B; the highest *Nematodirus* count recorded from any animal was 250 e.p.g. In the group A calves, *Nematodirus* eggs were passed principally during the first 8 to 10 weeks of the 1964 grazing season, while in the group B calves, *Nematodirus* eggs were recovered only during the latter part of the 1964 grazing season and the stabling period. In group C calves, *Nematodirus* eggs were recovered during the last week on the infected pasture.

The number and species of parasites recovered from the 23 animals are given in Table I. *O. ostertagi*, *C. oncophora* and *N. helvetianus* were the common parasites present, although a few *T. ovis* were also found, particularly in the group A yearlings. Fifteen to 30% of *O. ostertagia* recovered from the various groups of animals were immature. The number of immature *C. oncophora* and *N. helvetianus* ranged from 3 to 25% in different animals. The *C. oncophora* recovered from the group B yearlings in 1965 were generally smaller or "stunted" in comparison to those recovered from the group C calves (Fig. 4). Also, the female *Cooperia* from the group B yearlings contained fewer eggs than did those from the group C calves.

TABLE I. The Number and Species of Parasites Recovered from Experimental Animals when Exposed to an Infected Pasture as Calves, as Yearlings and as Both Calves and Yearlings.

| Group	No. of Calf	Days on infected pasture in 1964	Days on infected pasture in 1965	Date Examined	Ostertagia ostertagi	Cooperia oncophora	Nematodirus helvetianus	Trichuris ovis
A	1	39	0	7/8/64	2,320	31,540	1,400	0
	2	51	0	20/8/64	4,380	67,970	10,530	0
	3	51	0	20/8/64	6,420	109,640	9,170	0
	4	55	0	24/8/64	300	30,090	880	0
	5	62	0	31/8/64	1,080	46,660	0	0
	6	120	0	20/1/65	540	250	0	3
	7	120	28	21/7/65	1,790	30	0	1
	8	120	28	21/7/65	3,300	20	0	3
	9	120	28	21/7/65	3,130	0	0	0
	10	120	28	21/7/65	2,130	70	0	1
	11	120	28	21/7/65	13,730	0	0	3
	12	120	28	21/7/65	1,000	810	0	5
B	1	0	0	9/9/65	3	0	3	0
	2	0	28	21/7/65	6,800	72,010	0	0
	3	0	28	21/7/65	1,860	26,490	10	0
	4	0	28	21/7/65	7,140	14,160	0	0
	5	0	28	21/7/65	5,160	33,090	0	0
	6	0	28	21/7/65	2,400	121,640	4,840	0
	7	0	28	21/7/65	3,870	20,360	0	1
C	1	0	29	22/7/65	9,460	21,430	950	0
	2	0	29	22/7/65	4,140	58,870	8,870	0
	3	0	29	22/7/65	3,140	50,310	5,830	0
	4	0	32	25/7/65	6,430	99,040	750	0
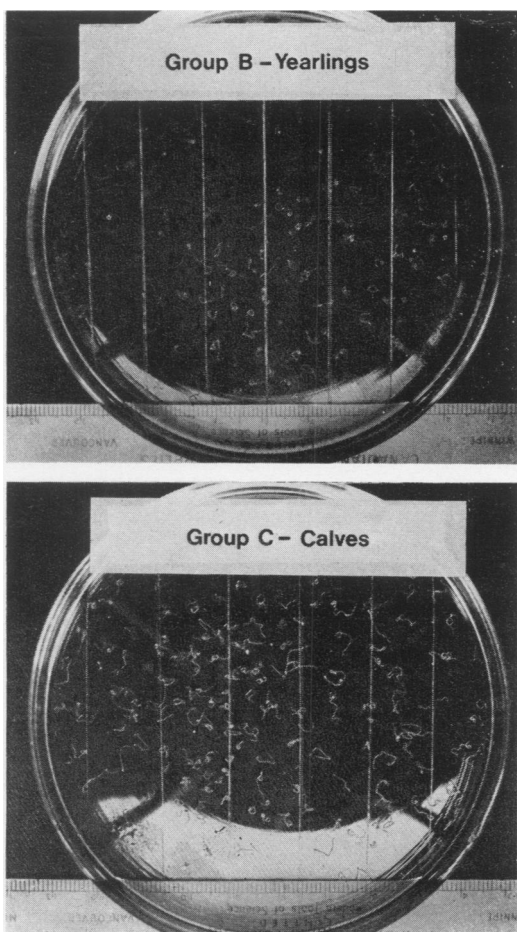
Fig. 4. Photographs showing the relative sizes of Cooperia oncophora from group B yearlings and group C calves killed 28 to 32 days after exposure to the same heavily infected pasture. One hundred specimens of each sex were chosen at random from each group. (Metric scale on ruler.)

DISCUSSION

The results of this experiment demonstrate the role that previous exposure may play in the epizootiology of C. oncophora infections in cattle. Calves that were examined within 8 to 9 weeks of exposure to a heavily contaminated pasture carried an average infection of over 57,000; other calves of the same group exposed at the same time, stabled overwinter and exposed again to the same pasture, were found to have few worms, average about 150, when they were killed and examined. The pasture was equally as infective the second year as the first, since parasite-free calves and yearlings about 15 months of age exposed

to it the second year had worm burdens comparable to those recorded from parasite-free calves the first year. The resistance to establishment of C. oncophora in older cattle would thus appear to be acquired rather than associated with the age of the host. This conclusion was also reached by Bailey (2) who found no evidence of an age-related resistance against C. punctata in calves up to 12 months of age.

The acquired immunity to C. oncophora which developed in the calves in this study as a result of exposure to infected pasture appeared to be very strong, as only token numbers of worms were recovered in the resistant animals as compared to the susceptible calves and yearlings. Furthermore, the serum of the resistant calves had higher complement-fixing titres with Cooperia antigen than those that were susceptible (9). Herlich (4) also observed that a strong immunity developed in calves when given single oral inoculations of infective larvae. Roberts et al (7) noticed that once a resistance against Cooperia spp. was established there were few instances of it breaking down later.

The poor development of Cooperia in the susceptible yearlings as indicated by their smaller size and fewer eggs when compared with those of susceptible calves, is not readily explained. Michel (6), working with Ostertagia in calves, has shown that there may be several distinct manifestations of resistance. While the older animals were shown not to have resistance to establishment of infection, it may be that once the parasites enter the host, other distinct and separate manifestations of resistance, such as retarded development, stunting and inhibition of egg laying, develop more rapidly than in young calves.

The development of resistance to N. helvetianus would appear to be less clearly defined than it was for C. oncophora. Four of 5 calves in 1964, and all 4 calves and 2 of 6 yearlings (one of which had only a few worms) in 1965, developed Nematodirus infections when exposed to a heavily contaminated pasture for the first time, while previously exposed yearlings were completely resistant. Unfortunately, the supposedly parasite-free yearlings had acquired very small Nematodirus infections when they were calves, as indicated by regular fecal examinations and the parasitological findings in calf B-1. Therefore, it is not known whether the failure

of 4 of the 6 yearlings to develop *Nematodirus* infections was due to age or to acquired resistance as a result of the extremely light infections. It should be pointed out that animal B-6, which developed an infection of almost 5,000 worms, did pass an occasional *Nematodirus* egg during the winter prior to going out to graze on the heavily infected pasture, indicating in this one instance, at least, that the previous light infection had not stimulated a resistance to infection. Obviously, also, evidence of age resistance to *Nematodirus* was not demonstrated by this animal.

Regardless of whether the age of the host is involved or not, animals which have been exposed to a heavy *Nematodirus* infection do build up a strong resistance and shed their worm burdens, as indicated in this experiment and in other investigations reported by the authors (11).

The *Nematodirus* burdens in those calves examined in 1964 between 39 and 62 days after exposure to infection were variable, ranging from 0 to over 10,000 worms. *Nematodirus* eggs were never recovered from calf A-5, which did not harbour *Nematodirus* at necropsy, so it is not known whether the resistance in this animal was inherent or acquired. It is possible that this animal did acquire an infection and had shed its worm burden prior to necropsy. The failure to recover *Nematodirus* eggs should not be taken as evidence that a *Nematodirus* infection was not established. For example, *Nematodirus* eggs were never recovered from calf A-3, yet this animal harboured over 9,000 worms. Fraser and Campbell (3) experimentally dosing calves of various ages with equal numbers of infective larvae, failed to demonstrate that age had a consistent effect upon resistance of calves to acquisition of infection by *N. helvatianus*, and suggested that the great individual variation in the size of the infection acquired was inherent in the calves.

The size of infections with *O. ostertagi* established in the various animals of this experiment for the most part fell within the range of 1,000 to 10,000 worms regardless of the age of the host or duration of the exposure to infection. The *Ostertagia* burdens and percentages of immature forms found in the various groups of calves suggest that, under the conditions of this experiment, there was very little evidence, if any, of a resistance to establishment of newly acquired larvae in the

yearlings exposed the previous year. Michel (6), Roberts *et al* (7) and Ross and Dow (8) have noted that resistance to this species is slow in developing. The findings in this study further suggest that the *Ostertagia* infections resemble those reported by Michel (6) in calves which were given daily doses of infective larvae. Self-cure, or the abrupt shedding of the *Ostertagia* worm burden, and a build-up of resistance to reinfection which presumably occurred for *Cooperia* and *Nematodirus,* as indicated by egg counts and/or necropsy findings, did not take place; instead, in consideration of Michel's findings (6) that worm populations are not static, the loss of worms presumably was continual with adult worms constantly being replaced. Under the conditions of this experiment, the various clinical manifestations of ostertagiasis, as reported in Scotland (1), did not occur.

The rapid drop in egg counts about 9 to 12 weeks after exposure to infection is undoubtedly associated with the build-up of resistance and the subsequent shedding of much of the *C. oncophora* (and *N. helvetianus*) worm burdens and concurs with the authors' earlier observations (11). The shedding of the worm burdens is also indicated by the rapid improvement of health and weight gains that occurred concurrently in surviving calves. Mayhew *et al* (6), working with *C. punctata,* noted that a rapid fall in egg counts was the first indication of immunity.

It is interesting to observe that a second and a third rise and fall in the fecal egg counts occurred in the group A calves. It is not known if these secondary and tertiary peaks in egg counts were due to reinfection or development of retarded larvae after the shedding of the initial adult worm burden. The tertiary rise noted in the late winter probably was due to development of retarded forms, since the conditions under which the animals were kept precluded the likelihood of reinfection. Unfortunately, it was not determined whether *Ostertagia* or *Cooperia,* or possibly both genera, were involved in the secondary and tertiary peaks of egg laying activity. Mayhew *et al* (5) reported that in the case of *C. punctata* egg counts rose and fell as many as three times before becoming low or negative.

With regard to egg laying activity, it is interesting to note that the egg counts recorded for the group A yearlings during the last eight weeks of the experiment were very low, yet each animal harboured

from 1,000 to almost 14,000 *Ostertagia* at necropsy. Michel (6) has shown that in calves given daily doses of *Ostertagia* larvae there is an inhibition of ovulation which prevents worms that develop later from becoming as prolific as those which developed earlier.

The low worm egg counts recorded for the group B yearlings in 1965, in contrast to the abrupt high rise in egg output observed for the group C calves with worm burdens of comparable size, are not readily explained. While the group B yearlings did not exhibit a resistance to establishment of worms, there did appear to be a marked retardation in development and inhibition of ovulation, as indicated by size of worms and egg counts, respectively. During the latter part of the first grazing season and the stabling period, the group B yearlings had been lightly infected, as indicated by fecal examinations and post-mortem examination of calf B-1. Serologically, the yearlings did show a weak activity with *Cooperia* antigen during this period (9). Thus, it would appear that the group B yearlings were sensitized by the light infections. The resistance which developed did not prevent establishment of infection but apparently was either strong enough or development was sufficiently rapid under the stimulus of the heavy infection to manifest itself by retarded development and inhibition of egg laying in the worms. Michel (6) regards the various manifestations of resistance as separate one from another and suggests each probably depends on different antigen-antibody reactions. While it seems less probable, the retarded development and inhibition of ovulation might also be associated with age of the host.

This experiment vividly demonstrates the adverse effect that heavy parasitism has on the development of susceptible animals. Calves that did not succumb to the infection became unthrifty and stunted as indicated by an average difference in weight of 128 lbs. between the yearlings that had been heavily parasitized as calves and those that had not. On the other hand, animals raised under conditions which allowed little or no exposure to parasitism were found to be much more susceptible to the effects of parasites than were previously exposed, resistant animals.

ACKNOWLEDGEMENTS

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