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STRAINS OF CATTLE PARASITES IN THE NETHERLANDS WITH DIFFERENT PROPENSITIES FOR INHIBITED DEVELOPMENT

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ABSTRACT

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A field study was undertaken of the possible differences in propensity for inhibited development in strains of cattle nematodes from two different locations in The Netherlands.

In one location (Lelystad) the strains were thought to lack the ability for inhibited development as a result of environmental stimuli on the infective larvae, while in the other location (Utrecht) this ability was presumed to be present. The Lelystad strains were kept at the original location and were also transferred to a pasture in Utrecht, while the Utrecht strains remained in Utrecht.

At both locations all permanent grazing calves showed high proportions of inhibited early fourth stage larvae of *Ostertagia ostertagi*, the proportion of the Utrecht strain being higher than the local and the transferred Lelystad strain. For *Cooperia oncophora* and *Nematodirus helvetianus* no clear differences between locations were observed in the permanently grazed calves.

No inhibition occurred in tracer calves turned out in August in either location, but differences were seen among tracer calves turned out in October. The Lelystad strains, kept in Lelystad or transferred to Utrecht, showed no inhibition, while in the tracer calves grazed on pastures contaminated by the Utrecht strains a marked inhibition of *O. ostertagi* and *N. helvetianus* was observed. The Lelystad strains appear to lack the ability, possessed by the Utrecht strains, to inhibit their development in autumn in response to environmental stimuli.

INTRODUCTION

Since the start of the extensive studies of Michel (1963) on *Ostertagia ostertagi* much attention has been paid to the phenomenon of arrested development. Anderson et al. (1965b) reported the presence of large numbers of *O. ostertagi* in the early fourth stage of development (EL-4) in tracer calves grazed for only 14 days in autumn. These results were confirmed by other

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observations (Anderson et al., 1965a; Armour et al., 1969a, b) and led to speculation on the existence of two different strains of *O. ostertagi*, one which was able to interrupt development (field strain) and another which was apparently less able to do so (laboratory strain) (Armour et al., 1967a, b; Michel, 1967; Sollod, 1967). Following these early observations much research has been carried out on several aspects of inhibited development, particularly in Glasgow and Weybridge. Besides environmental stimuli the development of host resistance has been shown to be a factor in the aetiology of inhibited development (Michel, 1974). The results of these investigations have been summarized by Michel (1974, 1978) and Armour (1978). The latter emphasized the similarity with diapause of insects (Armour and Bruce, 1974), which was later discussed by Horak (1981).

Recent studies by Smeal and Donald (1981) in Australia showed results which were sometimes at variance with earlier observations in Great Britain. They showed that after reciprocal transfer of parasite populations in calves from different geographical regions, no strain differences could be found with the exception of a significantly greater proportion of inhibited larvae in one of the populations of *O. ostertagi*. In later experiments Smeal and Donald (1982) demonstrated a consistently higher proportion of inhibition in a strain of *O. ostertagi* from beef cattle than in a strain from dairy calves kept on the same farm. The evidence for a genetic component in the inhibition phenomenon seemed to be clear. This idea was supported by results of other field experiments (Smeal and Donald, 1984) in which the progeny of previously inhibited worms showed a higher proportion of inhibited EL-4 than the progeny of worms which had developed directly.

In 1982 studies were carried out in The Netherlands to investigate possible strain differences in *O. ostertagi*. Earlier results obtained by one of the authors (Borgsteede, 1981) demonstrated that the *O. ostertagi* strain on the pastures of the Central Veterinary Institute at Lelystad only showed marked inhibition if calves were exposed to infection during the whole season (inhibition due to acquired immunity), whereas strong indications were obtained that the strain on the pasture of the Veterinary Faculty at Utrecht showed inhibition after exposure to environmental stimuli (physiological changes) as well (Eysker, unpublished data).

MATERIALS AND METHODS

Calves

Twenty-two Dutch Friesian male calves were used. They were bought at an age of 1—2 weeks, reared under wormfree conditions and turned out at an age of 3 months.

Experimental design

Three pastures were used. One pasture of 0.3 ha was located near Lelystad, the two others of nearly the same size were near Utrecht, 80 km away,

and climatic differences were regarded as negligible. The pasture in Lelystad had been grazed intensively since 1975 when a laboratory strain of *O. ostertagi*, isolated in 1971 and passaged 10 times, was taken outside. In later years *Cooperia oncophora* and *Nematodirus helvetianus* were also found on these pastures. One of the pastures in Utrecht had also been used for calves for more than 10 years with the same parasites occurring. The other pasture was not grazed by ruminants in the last 2 years before the experiment started and was regarded as free of the above mentioned species. On April 29, 1982, eight calves 3 months of age, were turned out in Lelystad and four of the same age were turned out in Utrecht on the first pasture. On May 18, four calves carrying the Lelystad strain of parasites were transferred from Lelystad and placed on the ungrazed pasture at Utrecht (Group II). One wormfree calf, 4 months of age, was added to the remaining Lelystad group on June 20, after two calves had died (on May 28 and June 10). All permanently grazed calves were housed on October 28 and slaughtered on November 18.

On August 26, groups of three tracer calves were placed on each of the three pastures (Groups IA, IIA, IIIA) and housed after 1 week of grazing. It was planned to slaughter these calves 21 days later, but during the housing period six calves died. On October 26 three further groups of three tracer calves were turned out again (Groups IB, IIB, IIIB). They were grazed for only 2 days, housed and slaughtered 21 days later. All tracer calves used in this study were reared free of parasites and were 3 months old when they were placed on the pasture.

Post mortem worm counts

The abomasa were slit open along the greater curvature and washed in a 15-l bucket. The fluid with the contents was mixed thoroughly and aliquots of 1/100 were collected, sieved (screenmesh 74 μm) and fixed with 4% formalin.

The abomasal wall was scraped off and digested for 4—5 h at 41°C in a pepsin—HCl solution. The digest was sieved, sampled (1/10) and fixed as described above. The contents of the small intestine were collected using a strong waterjet through parts of the intestine in a bucket. After concentration to a suitable volume by means of a sieve (screenmesh 74 μm) the above procedures for sampling were repeated.

Worms were counted and identified at low magnification (×16).

RESULTS

The results of the post mortem worm counts and the percentages of inhibited EL-4 are presented in Table I. (Permanent calves, Groups I, II and III; tracer calves, Groups IA, IB, IIA, IIB, IIIA and IIIB).

In Group I two calves died from parasitic gastroenteritis within 6 weeks of turnout. This was also the reason for the death of six of nine tracer calves turned out in August.

TABLE I

Post mortem worm counts for "permanent" and tracer calves

Group	Calf no.	Grazing period	Slaughter	*Ostertagia**				
				Adults	Dev. st.	EL_4	Total	% inh.[b]
I	2804	29/4—10/6	10/6[a]	31 700	0	0	31 700	0
	2812	29/4—28/5	28/5[a]	6 200	0	0	6 200	0
(Lelystad)	2808	29/4—28/10	18/11	6 040	1 470	12 170	19 680	61.8
	2810	29/4—28/10	18/11	30 100	7 110	43 950	81 160	54.2
	2844	21/6—28/10	18/11	16 000	1 890	16 110	34 000	47.4
								54.5
II	2803	29/4—18/5	18/11	44 480	5 300	175 320	225 100	77.9
(Lelystad-	2806	(Lelystad)	18/11	31 830	2 780	35 580	70 190	50.7
Utrecht)	2811	18/5—28/10	18/11	47 790	5 890	191 730	245 410	78.1
	2813	(Utrecht)	18/11	13 700	4 910	118 300	136 910	86.4
								73.3
III	94		18/11	6 800	400	87 500	94 700	92.3
(Utrecht)	130	29/4—28/10	18/11	13 990	200	77 000	91 190	84.4
	180		18/11	4 000	1 000	23 000	28 000	82.1
	183		18/11	11 450	1 100	74 700	87 250	85.6
								86.1
IA	2849	26/8—2/9	23/9	6 000	0	0	6 000	0
	2853		23/9	18 000	0	0	18 000	0
	2854		23/9	9 000	0	0	9 000	0
								0
IIA	2850		11/9[a]	117 900	53 900	500	172 300	0.3
	2851	26/8—2/9	14/9[a]	212 200	15 700	500	228 400	0.2
	2852		12/9[a]	112 000	58 900	200	171 100	0.1
								0.2
IIIA	2846		16/9[a]	47 600	100	800	48 500	1.6
	2847	26/8—2/9	16/9[a]	46 300	0	100	46 400	0.2
	2848		16/9[a]	45 600	0	100	45 700	0.2
								0.7
IB	2865		18/11	15 540	100	190	15 830	1.2
	2867	26/10—28/10	18/11	13 470	0	350	13 820	2.5
	2875		18/11	5 150	100	10	5 260	0.2
								1.3
IIB	2880		18/11	36 600	140	90	36 830	0.2
	2881	26/10—28/10	18/11	13 390	330	90	13 810	0.7
	2883		18/11	21 100	450	60	21 610	0.3
								0.4
IIIB	2862		18/11	6 680	740	7 850	15 270	51.4
	2866	26/10—28/10	18/11	11 290	200	9 480	20 970	45.2
	2870		18/11	5 180	110	11 160	16 450	67.8
								54.8

[a] Killed in extremis.
[b] Values in italics are means.
* mainly *Ostertagia ostertagi* (> 95%).
** mainly *C. oncophora* and *C. surnabada* (> 99%).
*** mainly *N. helvetianus* (> 99%).

*Cooperia***					*Nematodirus****				
Adults	Dev. st.	EL_4	Total	% inh.[b]	Adults	Dev. st.	EL_4	Total	% inh.[b]
0	0	0	0	—	0	0	0	0	—
40 000	0	0	40 000	0	200	100	0	300	0
0	0	0	0	—	0	0	0	0	—
13 900	0	0	13 900	0	0	0	0	0	—
3 300	0	0	3 300	0	0	0	0	0	—
				0					*0*
8 100	200	1 800	10 100	17.8	600	0	0	600	0
9 600	0	800	10 400	7.7	0	0	0	0	—
1 600	0	0	1 600	0	0	0	0	0	—
22 000	300	1 400	23 700	5.9	0	0	0	0	—
				7.9					*0*
6 400	0	8 000	14 400	55.6	0	0	0	0	—
0	0	3 000	3 000	100	0	0	0	0	—
0	0	0	0	—	0	0	0	0	—
25 900	0	54 900	80 800	67.9	0	0	0	0	—
				74.5					—
59 500	0	0	59 500	0	22 000	1 000	0	23 000	0
33 700	0	0	33 700	0	7 800	0	0	7 800	0
15 500	0	0	15 500	0	4 600	0	0	4 600	0
				0					*0*
172 000	139 500	1 500	313 000	0.5	0	0	0	0	—
463 000	133 500	0	596 500	0	4 000	0	0	4 000	0
177 500	3 500	500	181 500	0.3	6 500	0	0	6 500	0
				0.3					*0*
348 500	22 000	0	370 500	0	419 000	94 500	0	513 500	0
290 500	29 500	0	320 000	0	170 000	105 000	0	275 000	0
349 500	15 500	0	365 000	0	410 500	151 000	0	561 500	0
				0					*0*
44 700	200	0	44 900	0	5 300	0	0	5 300	0
43 500	0	0	43 500	0	2 100	0	0	2 100	0
22 600	0	0	22 600	0	1 100	0	0	1 100	0
				0					*0*
78 500	100	0	78 600	0	1 200	0	0	1 200	0
23 600	0	0	23 600	0	100	0	0	100	0
57 300	0	0	57 300	0	900	0	0	900	0
				0					*0*
24 600	0	0	24 600	0	40 600	3 300	55 200	99 100	55.7
60 000	0	0	60 000	0	113 900	1 700	53 100	168 700	31.5
22 800	0	0	22 800	0	29 500	600	45 700	75 800	60.3
				0					*49.2*

DISCUSSION

The results of the tracer calves (Groups IA, IB, IIA, IIB, IIIA and IIIB) clearly show that inhibition as a result of environmental stimuli was present only in the Utrecht strains of *O. ostertagi* and *N. helvetianus*, as for these species a high proportion of inhibited EL_4 was observed in Group IIIB. As this group grazed for only two days an effect of host resistance can be excluded. As almost no inhibited development was observed in Group IIIA, grazing in August, these environmental stimuli would appear to be related to autumn conditions, as they are in Great Britain (Anderson et al., 1965a, b; Armour et al., 1969a, b). Such an influence of environmental stimuli was not observed in the Lelystad strains nor in the Utrecht strain of *C. oncophora*. For the Lelystad strains the transfer to Utrecht did not change this.

In all permanently grazed groups high levels of inhibited EL_4 were observed for *O. ostertagi*. The proportion of inhibited EL_4 was lower in Group I than in Groups II ($P < 0.1$) and III ($P < 0.01$) (Student's *t*-test). In the latter group the proportion of *O. ostertagi* EL_4 was higher than in Group IIIB ($P < 0.05$). These results imply for both groups originating from Lelystad that inhibited development was probably the result of development of host resistance. The higher proportion of inhibited EL_4 in Group II compared with Group I could be explained by the higher infection rates at the end of the grazing season, which is clearly shown by the worm counts of the Groups IIA and IIB compared with those of the Groups IA and IB. The high levels of inhibition of *O. ostertagi* in Group III may be explained by the effect of host resistance superimposed on the effect of environmental stimuli. High levels of inhibition for *C. oncophora* were only observed in Group III. As in the case of *O. ostertagi* in Groups I and II, these high levels cannot be explained by environmental stimuli, as no inhibition was observed in tracer calves, but they may be explained by the development of host resistance. However, comparison of the numbers of worms in the Groups I and II with those in the Groups IA and IB and in IIA and IIB, respectively, strongly indicates development of resistance to *C. oncophora* in these groups, particularly in Group II. Nevertheless, only low levels of inhibition were seen in Group II for *C. oncophora*. The difference between Group II and Group I, i.e. low levels of inhibition compared with no inhibition, may be explained by a higher reinfection in Group II shown by higher *C. oncophora* counts for Groups IIA and IIB compared with IA and IB. The differences between Groups II and III cannot be explained by differences in infection levels, as these are not shown by the worm counts of the tracer calves. Possibly the Utrecht strain is more inhibition prone than the Lelystad strain, though this was not expressed in the tracer calves. *N. helvetianus* evokes a rapid and strong host reaction compared with *C. oncophora* and particularly with *O. ostertagi*, as was concluded from patterns of egg output (Borgsteede, 1977). Therefore almost no worms were found in the permanent grazing calves. This also implies that the epidemiological significance of a seasonal

pattern of inhibition, as observed for the Utrecht strain is of minor importance, as young cattle become refractory to this species relatively quickly and resumption of development followed by egg output will be very rare. Moreover, the free living stages of this species can survive easily (Rose, 1975).

The most interesting aspects of the work of Smeal and Donald are that they showed higher levels of inhibition of *O. ostertagi* in beef calves than in dairy calves grazed on the same farm (Smeal and Donald, 1982) and that levels of inhibition in the progeny of formerly inhibited worms were higher than in the progeny of worms which developed directly (Smeal and Donald, 1984). Their explanation for the differences between beef and dairy calves was that in the latter inhibited larvae contribute very little to the survival of the worms from year to year, whereas in beef calves this is not the case. Consequently a higher selection pressure for inhibited development occurs in beef cattle than in dairy cattle. Their later results confirmed that such selection can be effective (Smeal and Donald, 1984).

Selection for inhibition did not occur in either of the Lelystad strains or in the Utrecht strains as the strains were maintained on pasture by calves. Thus only the progeny of worms which developed directly contributed to pasture contamination. This may explain why both *C. oncophora* strains and the Lelystad strain of *O. ostertagi* show a low ability to inhibit development as a result of autumnal stimuli, compared with the results from Great Britain (Michel et al., 1974). The differences between the two *O. ostertagi* strains may be related to differences in history. The Lelystad strain was isolated from the field in 1971 and passaged 10 times until it was returned to pasture in 1975. The Utrecht strain was brought on pasture with naturally infected calves in 1968. In the Lelystad strain inhibited development was never seen in tracer calves. This strain may have lost the propensity for inhibited development, in response to environmental stimuli, during these passages indoors as has been seen to a lesser extent in other laboratory strains (Armour et al., 1967a, b; Michel, 1967). However, it is also possible that the Lelystad strain never possessed this ability. Michel (1974) did an experiment in 1970 with a Weybridge strain of *O. ostertagi* which had approximately the same history as the present Lelystad strain, as it had been isolated from the field in 1957 and had been passaged indoors until it was brought to pasture in 1964. In contrast to the Lelystad strain, this strain showed inhibition of development as a result of environmental stimuli though the levels of inhibition were lower than in Glasgow and Weybridge field strains. An *O. ostertagi* strain which behaved similarly to the Lelystad strain was described in New Zealand (Brunsdon, 1972).

The present experiments clearly show that a variation in the ability of *O. ostertagi* to inhibit development can be demonstrated in The Netherlands. The epidemiological role of the phenomenon is not clear and may vary considerably between farms, depending on factors such as the separate grazing of calves and older cattle, the use of anthelmintics, particularly at housing. A seasonal pattern of inhibition has been previously demonstrated in The

Netherlands in naturally infected adult cows from different sources (Borgsteede and van der Burg, 1982). This does not necessarily mean that the situation observed in the Utrecht strain is more representative of the Dutch situation than that in the Lelystad strain. This seasonal pattern of inhibition of *O. ostertagi* in cows may very well reflect a seasonal pattern of host resistance, such as is indicated by the results of Kloosterman (1983).

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