

VARIABILITY OF RESISTANCE OF CALVES TO ACQUISITION OF INFECTION BY *NEMATODIRUS HELVETIANUS**

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Nematodirus helvetianus infection is common in Ontario calves, and occasionally appears to cause severe clinical signs. Clinically affected calves are usually smaller than others with which they are associated. If these observations are valid a number of factors could be responsible: (1) Younger calves may be more susceptible to infection and less resistant to its effects. (2) Smaller calves, less successful in the competition for available feed and consequently malnourished, have a lower resistance to infection and its effects. (3) Because of the unsuccessful competition for feed, smaller calves may be compelled to pick over what feed is left and thereby expose themselves to the ingestion of larger numbers of infective larvae. (4) A combination of these factors may be significant. (5) It is also possible that the apparent causes are not the true ones and that some other factor, perhaps inherent, determines which calves become infected.

Evidence supports the existence of true age resistance to helminthiasis as well as that due to acquired immunity, although in some other instances age resistance appears to be absent (12). Older animals are apparently able to resist the effects of parasitism better, but there is doubt in some cases at least, regarding true age resistance to acquisition of infection.

Brunsdon (3) demonstrated what appeared to be an age resistance in sheep that was stronger against infection by *Nematodirus filicollis* than by *N. spathiger*. This suggested that there are specific biological differences and that for particular host-parasite systems, including calf-*N. helvetianus*, one must rely on specific investigations and not generalize.

Little information concerning this particular system and the effects of age of the host has been reported in the literature. Acquired immunity or age resistance to *N. helvetianus* was clearly demonstrated in one calf. In other calves in the same study there was some correlation between length of the prepatent period and the age of the calves, suggesting age resistance (11). Adult cattle have been reported to be resistant (12).

The effect of nutrition on the resistance of the host to infections is not clear. In most instances, however, it appears that a deficient diet favors the establishment of helminth populations (13). In addition to the possible direct effects of nutrition on resistance to, or the effects of, infection, animals on short pasture are forced to eat more of the plant stem and may thus acquire greater numbers of larvae (22, 23). On the other hand, the more herbage an animal eats the more larvae it may be expected to ingest (12, 21).

A lowering of true age resistance to acquisition of infection by *Nematodirus* spp. was demonstrated in 18-month-old lambs which were severely restricted in quality and quantity of feed for a period of four months prior to being put on a heavily contaminated pasture (4). These lambs acquired six times as many worms as did a well-fed control group of the same age, and only a few worms less than well-fed, six-month-old lambs.

The present study was designed to evaluate the effects of age and nutrition on the acquisition of *N. helvetianus* by calves.

MATERIALS AND METHODS

Feces were collected daily from calves naturally infected with a mixture of gastrointestinal helminths, and from calves artificially infected with *N. helvetianus*. The *Nematodirus* spp. eggs were then obtained in pure culture from the feces by a modification of the methods of Wright (28), Seghetti (19), and Thomas (24).

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In order to retard bacterial fermentation, the eggs, plus some inseparable debris, were stored at a maximum of 10° C. in water containing a few drops of formalin, until required for incubation.

The eggs were hatched by a modification of the methods of Seghetti (19), and Thomas (24), and the larvae recovered from the debris by a modification of the Baermann technique. The larvae were then stored in shallow water at 3° C. until used to infect the calves.

Calves were obtained at one to three days of age and raised on concrete in an attempt to keep them free of internal parasites. Until the time of experimental dosing, the calves were kept in pens, well bedded with straw or shavings. Each pen contained one, two, or four calves. For the duration of the experiment, 30 calves were tied in individual stalls; these were all housed in one building containing 30 individual tie stalls separated by a solid partition. All stalls were cleaned daily. The calves were from four to 50 weeks of age at the time of administration of larvae. From one month prior to the administration of larvae until the end of the experiment, the calves were divided into two groups paired as to ages. One group of 15 was fed an inadequate diet, while the other was put on a high plane of nutrition (Appendix I). The calves were stabled in random order.

The feces of all calves were checked periodically for parasites. Sporadically a few coccidial oocysts were found, on a few occasions a few *Strongyloides* spp. eggs, and on one occasion a *Trichuria* spp. egg. Five calves developed low grade infections with *Nematodirus* spp. prior to the experimental administration of *N. helvetianus* larvae and these were withdrawn from the experiment. The apparent source of this infection was determined but no other parasite-free calves of suitable ages were available and the experiment was continued using 25 calves. All of the calves were castrated male Holstein-Friesians except four female Holstein-Friesians and three castrated males of other dairy breeds.

EXPERIMENTAL TECHNIQUES

One month after the calves had been placed on the experimental diets, each

calf was given 10,000 infective *N. helvetianus* larvae which had been stored one week. All larvae were from the same culture and had been divided into lots less than an hour before their administration as a drench.

Feces from all 25 calves were checked for parasite eggs on days 1, 4, and 11, and daily from day 13 until the end of the experiment. Differentiation was made between mature and immature stages, and all males with spicules and all females containing eggs were classified as adults.

OBSERVATIONS AND RESULTS

Following administration of larvae, an egg was recovered from one calf on day 13, from two other calves on day 20, and from all but three of the calves by day 30. Adult worms were recovered from all calves including these three. The prepatent periods were unrelated to size of worm burdens or to age or nutritional status of the calves.

The number of eggs recovered per sample varied from one to 80, and in all cases was variable from day to day. There was some tendency for the calves, which later proved to have the largest worm burdens, to pass more eggs than those carrying fewer worms but this was not consistent. There was no discernible relationship between numbers of eggs passed and age or nutritional status of the calves.

Numbers of mature and immature worms which were recovered were recorded and have been compared in two different ways in graphic form (Figures 1 and 2). The total numbers of worms recovered per calf varied from 70 to 2,140, while the ratio of mature to immature worms varied from 1,510:0 to 20:50. Neither the total numbers of worms recovered, nor the ratios of mature to immature worms, fell into a pattern which could be related either to age or to nutritional status of calves.

Both the female calves and the male calves of other breeds fell within the range covered by the Holstein-Friesian steers with regard to prepatent periods, numbers of eggs passed and worm burdens. Although actual measurements were not taken, no difference in sizes of mature worms were noted.

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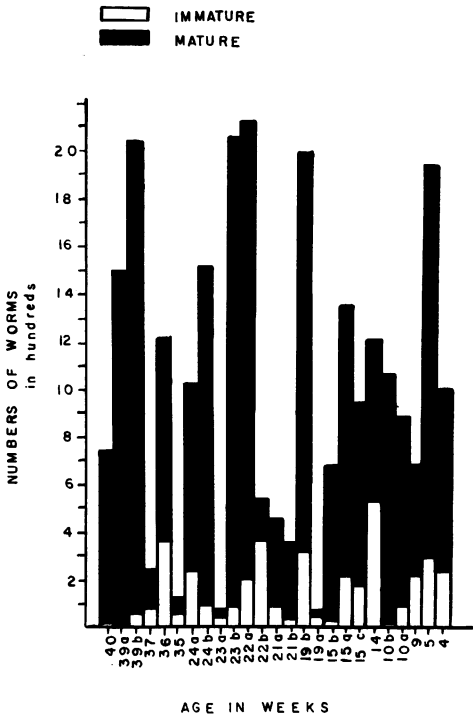


FIGURE 1. This illustrates the number of mature and immature *N. helvetianus* found at necropsy of 25 calves of varying ages fed at one of two nutritional levels, 31 days after administration of 10,000 larvae per calf. The calves are arranged in order from oldest to youngest without regard to nutritional status.

DISCUSSION

In this study there is no evidence that resistance to infection is influenced by age. Under the conditions of this experiment, malnutrition did not lower the resistance of the calves to acquisition of infection with *N. helvetianus*. The inadequate diet, although not analyzed, was obviously low in protein, vitamin A and carbohydrates. The period of semi-starvation was not protracted since the ages of some of the calves precluded the exertion of more severe stress. It is difficult, however, to imagine that young calves could be more poorly fed.

In hemonchosis it is possible by severe stress of malnutrition to break down the resistance of some sheep, but not others to hemonchosis (17). This led to the suggestion that natural or genetic resistance remained in some animals and was not re-

duced by starvation. Although a clear distinction was not made, it is fairly obvious that this reduction was in resistance to the effects of an established infection. It is possible, however, to speculate that in the present investigation natural or genetic resistance to the acquisition of infection was demonstrated, thus providing an explanation for the results.

It is a common experience that under the same conditions of exposure some animals of a group succumb to illness while others do not. There is no question of the existence of inherent variability of resistance to infectious agents between host species, and little of its existence within species. There are several references in the literature which indicate the existence of such inherent variability of resistance to various parasitic infections (5, 6, 7, 10,

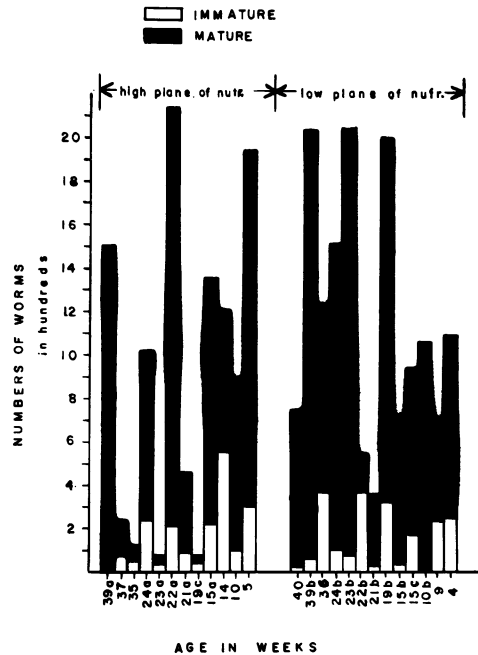


FIGURE 2. This illustrates the number of mature and immature *N. helvetianus* recovered at necropsy of 25 calves of varying ages and fed at one of two nutritional levels, 31 days after the experimental administration of 10,000 larvae per calf.

The calves are arranged in two groups, according to their nutritional status, low plane or high plane, and within each group the calves are arranged in order from oldest to youngest.

15, 16, 17, 18, 25, 26, 27), and a few to an apparent variability in sheep with regard to their natural resistance to nematodirosis (1, 2, 9). In these reports, however, the authors appeared to consider that the variability was due chiefly to inability of the hosts to resist the effects of infection (1, 2, 9).

Incidental observations within the context of reports on other patterns of resistance are perhaps more numerous than direct references to individual variation. In one study on the effect of age on resistance of cattle to gastrointestinal helminth infection, considerable variation was noted within age groups (12). It was suggested that these differences may be related directly to the difference in amount of forage consumed or to chance grazing of more or less heavily contaminated parts of the pasture. The possibility of inherent lack of resistance was not mentioned as a probable explanation of the differences.

In two other studies of experimental nematodirosis in sheep, tabular summaries of results revealed a considerable variation in the number of worms recovered from lambs receiving identical numbers of infective larvae (8, 20). These variations could not be related entirely to the age of the lambs.

Brunsdon (4), although able to correlate age with resistance of sheep to *Nematodirus* spp., also recorded considerable variation within the age groups. Admittedly, these lambs were naturally infected and there are therefore other possible reasons for the variations in the numbers of worms recovered at slaughter. Nevertheless, the range of 5,700 to 45,500 in the 18-month-old group and 6,200 to 64,800 in the six-month-old group is interesting. There was, however, considerably more uniformity within the groups, and a great deal more difference between them in Brunsdon's experiment than in those of this study. Under conditions of natural infection at pasture in Brunsdon's experiment, one would expect greater variations.

In this experiment no breed difference in resistance of calves to *N. helvetianus* was observed although it is impossible to draw a valid conclusion because of the numbers of animals used (one each of three breeds other than Holstein-Friesian). In this investigation, it is apparent that the

main variable in the factors modifying resistance of the calf to *N. helvetianus*, is inherent in the calves. The discovery of naturally acquired low grade infections in five of the original 30 calves introduces the possibility that other similar infections occurred but were missed. Such infections could have led to varying degrees of immunity before the experimental dosing. Only a repetition of the trial could unequivocally answer the question.

SUMMARY AND CONCLUSIONS

Twenty-five calves, divided two ways on the basis of age and nutritional status, were given identical numbers of *Nematodirus helvetianus* larvae. Egg counts were made daily from the 12th to the 30th day after administration of the larvae, after which the calves were sacrificed. Counts were made of all worms, mature and immature, recovered from the small intestine at necropsy. Prepatent periods, egg production (as measured by the egg counts) worm counts, and worm sizes were compared.

Under the conditions of this experiment neither age nor nutritional status had a consistent effect upon resistance of calves to acquisition of infection by *N. helvetianus*.

There was great individual variation in the size of the infection acquired. It is suggested that this apparent variability in resistance was inherent in the calves.

RÉSUMÉ

Vingt-cinq veaux répartis en deux groupes suivant leur âge et leur état de nutrition ont reçu le même nombre de larves de *Nematodirus helvetianus*. Des énumérations d'œufs ont été faites quotidiennement du 12ième au 30ième jours qui ont suivi le début de l'expérience. Les veaux furent abattus et le dénombrement total des vers a été fait au moment de l'autopsie. L'énumération des œufs et des vers ainsi que les mensurations de ces derniers furent comparées en tenant compte des périodes de développement postembryonnaire chez les hôtes.

Selon cette expérience, ni l'âge, ni l'état de nutrition n'ont influencé le processus de résistance à l'infection causée par *N. helvetianus*.

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On a constaté des variations individuelles considérables dans l'intensité de l'infection. On croit que ces variations sont dues à la résistance naturelle de chacun des veaux.

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APPENDIX I

Diets Fed Calves in an Experiment Evaluating the Effect of Nutrition on the Acquisition

by Calves of *Nematodirus helvetianus* Infection

Both diets were fed for the duration of the experiment commencing one month before the administration of larvae to the calves.

Low Plane of Nutrition

All these calves were given water and either straw or very poor-quality, three-year-old hay. The young calves, in order that they might not die of nutritional inadequacies, were given milk twice daily until they reached seven weeks of age. Three calves just over

seven weeks of age were also given very limited quantities of pelleted calf feed.

High Plane Nutrition

These calves were given water and good quality hay *ad libitum*. All calves were given milk twice daily until they were ten weeks of age and up to 20 weeks of age were given a commercial pelleted calf feed *ad libitum*; calves over 20 weeks of age were twice daily given a liberal amount of a mixture of ground grains plus a commercial vitamin-mineral concentrate.

ABSTRACTS

Parikov, V. A. (1965). (Treatment of bovine mastitis with ultrasound). Veterinariya, Moscow 42, No. 6 pp. 77-81 (R.) (Sel'skokhoz. Institut, Voronezh, USSR).

Acute catarrhal mastitis was induced in 30 cows by intramammary injection of *Staphylococcus aureus*. Twenty were treated with ultrasound: after the udder had been washed with soap followed by ethyl alcohol, then lubricated with 50% aq. soln. of glycerine, an ultrasound emitter (880 kilohertz) was rubbed lightly over the skin at a speed of 1-1.5 cm./sec. Best results were achieved with a daily ten-min. treatment at 0.6-0.9 watts/cm.² until recovery. Ultrasound at high intensity (up to 3 watts/cm.²) aggravated severe mastitis. Preliminary application of 2% tetracycline soln. to the skin of the udder had no significant effect. The therapeutic effect of daily injection of tetracycline into the udder in ten cows was inferior to that of ultrasound.

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Straub, O. C., and Kielwein, G. (1965). (Bovine enteroviruses as agents of mastitis.) Berl. Münch. tierärztl. Wschr. 78, 386-389 (G.e.) (Bundesforschungsanst. für Viruskrankh. der Tiere, Tübingen, Waldhäuser Höhe).

Details were given of experimental intramammary infection of two cows with fresh tissue culture suspension of CBV-D virus

(V.B. 35, abst. 3821), which resulted in acute catarrhal mastitis with increased cell count. The virus was re-isolated from milk of infected quarters. Both cows recovered after about 14 days.

Reprinted from "The Veterinary Bulletin", Vol. 36, No. 4, April, 1966.

Lesslie, I. W., and Hebert, C. N. (1965). The use of dilute tuberculins for testing cattle. Br. vet. J. 121, 427-436 (Central Vet. Lab., Weybridge).

The doses of tuberculin at present used for testing cattle are large. A pilot trial was carried out in experimentally sensitized cattle to determine whether the differentiation of specific and non-specific sensitivities is likely to be easier at low concentrations of tuberculin, and also to compare skin thickening and diameter of induration as measures of the tuberculin reaction. Results indicated that the specificity of the test is not increased with dilute tuberculins. Correlation between skin thickening and induration was highly significant, and the larger the reaction the better the correlation. Reactions to tests with large doses of tuberculin given immediately after reading the 72 hour reactions to a previous set of injections were greatly depressed.

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