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Effect of Strategic Treatments with Invermectin on Parasitism of Set-Stocked Calves Exposed to Natural Trichostrongyle Infection in Lithuania

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[1]Department of Infectious Diseases, Lithuanian Veterinary Academy, Kaunas, Lithuania, [2]Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark, [3]Food and Agriculture Organization of the United Nations, Rome, Italy.

Šarkūnas M, Malakauskas A, Nansen P, Hansen JW, Paulikas V: Effect of strategic treatments with ivermectin on parasitism of set-stocked calves exposed to natural trichostrongyle infection in Lithuania. Acta vet. scand. 1999, 40, 163-172. – The effect of strategic treatments with ivermectin in first-season calves exposed to trichostrongyle nematodes on naturally contaminated pasture was studied. Twenty first season heifer calves were divided into 2 groups, according to live weight, and on 22nd May each group was turned out onto a 1 hectare pasture. Group A (Plot A) was treated with ivermectin at weeks 3, 8 and 13 after turn out, while group B (Plot B) served as an untreated control group. The study showed that control calves exhibited increase in trichostrongyle egg counts in August, while treated calves were excreting low numbers of trichostrongyle eggs. Pasture larval counts on Plot B (control animals) were low during the first part of the grazing season, followed by a steep rise towards the end of July. In contrast, the numbers of infective larvae recovered from Plot A remained low throughout the season. Both groups showed comparable weight gains from May up to the middle of July. However, from then on, Group B (controls) had lower weight gains than ivermectin treated Group A. From the end of July onwards, most untreated calves (Group B) showed clinical signs of parasitic gastroenteritis. It can be concluded that the strategical ivermectin treatments were successful, and faecal egg counts, pepsinogen levels and herbage larval counts clearly demonstrated that this was accomplished through suppression of pasturc contamination with nematode eggs and subsequent reduction of pasture infectivity.

trichostrongyles; pasture; strategical treatments; parasite.

Introduction

Ostertagia ostertagi is the most pathogenic and economically important nematode parasite in first-season grazing calves in Lithuania. Clinical outbreaks of parasitic gastroenteritis usually occur in the second part of the grazing season following a build up of infective larval populations on the herbage, as recently observed by *Šarkūnas et al.* (1998).

Original studies in England showed that, infective larvae survive on pasture throughout the winter (*Rose* 1961) to become the source of infection for calves turned out to graze in spring or early summer. This early infection results in recontamination of the pasture with nematode eggs as described by *Michel* (1969) and a subsequent increase in the number of infective larvae on herbage from mid-summer through autumn occurs. A similar herbage infectivity pattern has been recorded in other countries influenced by the Atlantic climate, e. g. Germany (*Bürger* 1966), Sweden (*Nilsson & Sorelius* 1973) and Denmark (*Henriksen et al.* 1976, *Nansen* 1993). However, in Lithuania, no data have been published on the seasonal fluctuations in herbage infectivity and epidemiology of parasitic gastroenteritis in first-season grazing calves until a recent observation by *Šarkūnas et al.* (1998). Nevertheless, a survey by *Čygas* already in 1957, on the occurrence of gastrointestinal nematodes in Lithuania showed that the highest burdens of nematode parasites in first-season grazing calves were observed in the beginning of Autumn. However, no strategies for control of trichostrongyle infections have yet been advocated in Lithuania. *Šarkūnas et al.* (1998) describing the late season build-up of infective larvae on pasture showed that a mid-summer move of calves to pasture free from parasites diminished the risk of clinical disease. Lithuania has a climate intermediate between maritime and continental ones. Rather cold winters, characterised by low temperatures and a constant snow cover, prevail. This may favour the survival of the larvae (*Michel* 1978) and may help to explain the fact that overwintered larval populations may persist on the pastures in rather high numbers until early summer (*Šarkūnas et al.* 1998) On this background it seems necessary to determine the epidemiological pattern of trichostrongyle infections separately for Lithuania and to work out control strategies on the basis of this pattern.

A number of anthelmintic control principles has been recommended to control parasitic gastroenteritis in first-season grazing calves, which is the age group most susceptible and often suffering severe production losses due to trichostrongyle infections (*Henriksen et al.* 1976, *Nansen* 1993). One of these is an early-season strategical anthelmintic treatment scheme to lower egg contamination and hence preventing the build-up of high pasture infectivity during the late summer. The beneficial effect of anthelmintic treatments at weeks 3, 8, and 13, using ivermectin with its highly persistent activity, has been demonstrated in e.g. Northern Ireland (*Taylor et al.* 1985), Scotland (*Armour et al.* 1987), England (*Ryan et al.* 1986), Belgium (*Hollanders et al.* 1992) and Denmark (*Satrija et al.* 1996). The timing of the treatments is based on the prepatent period of trichostrongyles of approximately 3 weeks and the persistent effect of the ivermectin against these nematodes of approximately of 2 weeks (*Armour et al.* 1985). The 3 consecutive treatments are needed when grazing season is longer and pasture challenge is heavier. Such a strategy can provide benefits in both the early and later stages of the year and reduces the risk of type II ostertagiosis (*Jacobs et al.* 1987; *Hollanders et al.* 1992).

The present investigation, carried out in Lithuania, describes the natural trichostrongyle infections on permanent pasture in 2 separate groups of first-season grazing calves. One of the groups was treated with ivermectin using the above mentioned strategic dosing program while the other group was left untreated.

Materials and methods

Animals and experimental design

The experiment was carried out during the grazing season of 1996 on a private dairy farm situated 59 km east of Kaunas, Kaišiadorys District, Lithuania. A permanent pasture of 2 hectares grazed by young stock infected with trichostrongyles (*Ostertagia* spp., *Cooperia* spp. and *Nematodirus* spp.) the preceding year was divided into 2 comparable plots each of one hectare.

Twenty first season grazing heifer calves aged 5-8 months that were not previously exposed to trichostrongyle parasites were turned out on

22nd May, 1996. They were cross-breeds between the Holstein/German Black Pied and local Lithuanian breeds. According to live weight, the calves were divided into 2 comparable groups of 10 animals, Group A (Mean weight ± SD = 122 ± 26 kg) and Group B (Mean weight ± SD = 122 ± 36 kg). Calves in Group A were treated at weeks 3, 8 and 13 after turnout, while animals in Group B served as an untreated control group. Each treated animal was given the recommended dose of 200 μg/kg of body weight of ivermectin subcutaneously (Ivomec®, MSD-AGVET). To compensate for the gradually declining grass growth naturally occurring during the season in Lithuania, the grazing area of each group was extended to incorporate an additional ½ hectare of aftermath per group on 10 August. All animals were housed on 16 October.

Parasitological analyses
Blood and faecal samples were obtained fortnightly, and on the same occasions herbage samples were collected for determination of the numbers of infective trichostrongyle larvae. The faecal egg counts expressed as eggs per g of faeces (EPG) were performed using a modified McMaster technique (*Henriksen & Aagaard* 1976). For identification of infective third-stage larvae at genus level, bulk faecal samples were obtained from each group every month, and faecal cultures were established according to the method described by *Henriksen & Korsholm* (1983). Herbage samples were collected from each experimental plot fortnightly following a W-shaped route across each plot. The grass was collected manually stopping at intervals of 10 steps. Grass within 20 cm of dung pats was avoided in order to imitate the natural grazing behaviour of cattle. Each sample consisted of 200-300 g of herbage. Infective trichostrongyle larvae were isolated using an agar gel technique established by *Mwegoha & Jørgensen* (1977) and subsequently counted, differentiated and expressed as numbers of larvae/kg of dried grass. Levels of serum pepsinogen were determined according to the procedure described by *Ross et al.* (1967). According to this method the acid activation of pepsinogen to pepsin in blood serum was used. Hereby peptides were obtained, containing tyrosine ens groups. Folin-ciocalteau reagent yields, with the tyrosine groups, the blue colour which is proportional to the pepsinogen concentration in blood serum.

Other observations and statistical analyses
On each sampling day (2-week intervals), calves were weighed in a portable scale and clinical inspections were performed. Data on monthly precipitation and daily average temperatures were obtained from a meteorological station situated 17 km from the pasture. For comparison, data for precipitation and average daily temperatures in the period 1961-1990 were obtained from the Kaunas Meteorological Station. During the experiment, the amount of grass available in the experimental plots was estimated by visual inspection.
Faecal egg counts (log-transformed according to log (x + 1)), body weight and blood serum pepsinogen levels (log-transformed) were analysed by repeated measures analysis of variance (ANOVA), using SAS version 6.08 (Statistical Analysis Systems, 1991). This tested for the between-group effect of treatment, the within-animal effect of time and the time-group interaction.

Results

Herbage larval recovery and faecal egg counts
The numbers of infective trichostrongyle larvae on herbage were moderate to low and comparable on both experimental plots until the middle of July (Fig. 1). From then onwards the num-

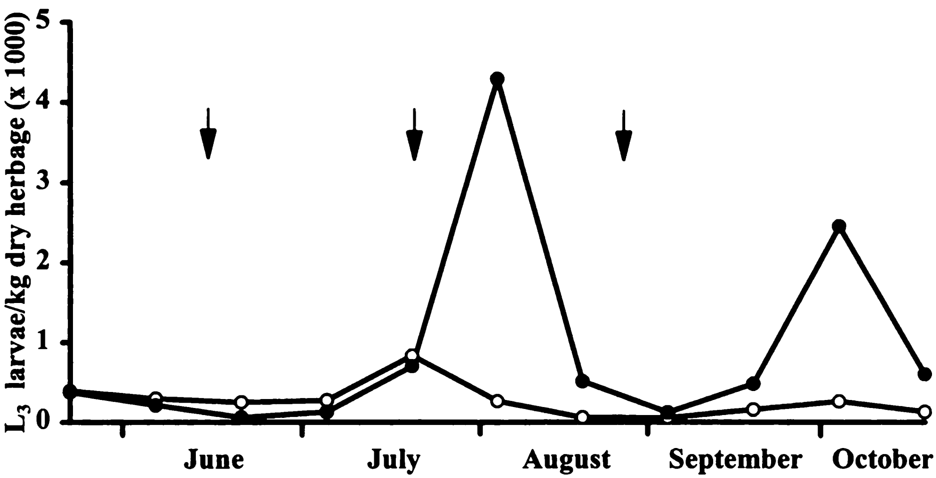

Figure 1. Mean herbage trichostrongyle larval counts for 2 experimental plots. (○) represents Plot A where treated calves were grazing. (●) represents Plot B where control calves were grazing. Arrows indicate the time of strategical treatments.

bers of larvae exhibited a steep rise on Plot B up to more than 4000 larvae/kg of dry herbage, while numbers of larvae on Plot A remained around 200 larvae/kg of dried herbage throughout the rest of the grazing season. A second rise in numbers of larvae on Plot B was observed towards the end of September.

Trichostrongyle egg counts (Fig. 2) were rather low to moderate in the non-treated control Group B in June-July and did not exceeded 100 epg. Subsequently, they rose gradually during late July and August to reach a peak value of approximately 250 epg. In contrast, egg counts in the treated Group A remained low (less than 16 epg) after the first treatment throughout the rest of the season. When tested by repeated measures analysis of variance there was a significant difference between groups ($p<0.001$). Results from faecal cultures showed roughly comparable numbers of *Ostertagia* spp. and *Cooperia* spp. in the non-treated Group B, while *Cooperia* spp. were only species found in the faecal cultures from treated calves (Group A). *Nematodirus* spp. eggs were sporadically found in small numbers in both groups at the start and the end of the grazing season. Other nematode eggs occasionally found in the faeces of the control animals were *Strongyloides* spp. and *Trichuris* spp.

Serum pepsinogen levels

At the start of the grazing season serum pepsinogen levels (Fig. 3) were low in both groups. From the end of June, however, mean pepsinogen levels rose gradually in the non-treated Group B to reach a peak value of around 1,8 IU tyrosine/litre towards the end of August, while the levels in the treated Group A remained low and within the normal range (0.5-0.8 IU tyrosine/litre) throughout the grazing season. When tested by repeated measures analysis of variance there was a significant difference between groups ($p<0.001$).

Performance and clinical observations

Mean cumulative weight gains of Group A and

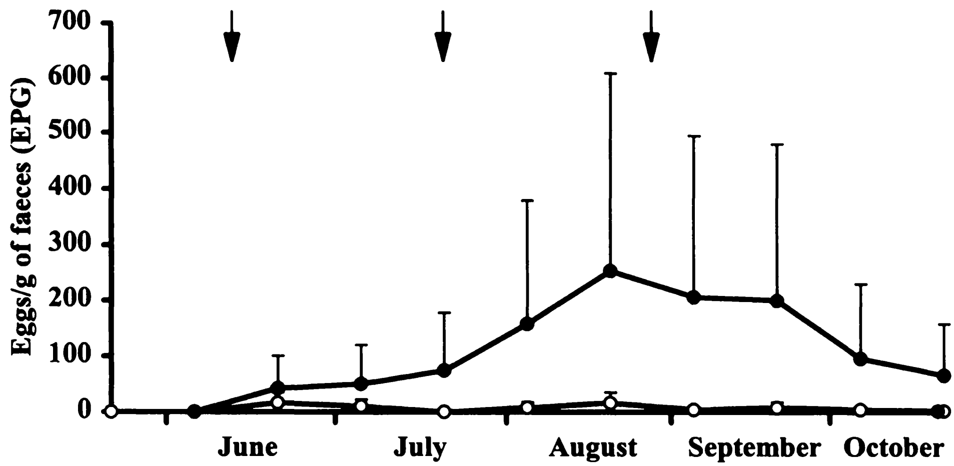

Figure 2. Mean faecal trichostrongyle egg counts ± standard deviation for 2 experimental groups of calves. (○) represents Group A of calves which were treated with ivermectin at week 3-8-13 after the start of the grazing season. (●) represents control Group B of calves. Arrows indicate the time of strategical treatments.

B are presented in Fig. 4. Weight gains were increasing throughout the experiment, however, the treated Group A exhibited higher weight gains than Group B from July onwards. At housing, calves in Group A had an average live weight of 229.2 ± 53.4 kg compared to average live weight of 201.4 ± 59.5 kg of those in Group B. In the end of August eight of ten non-treated calves (Group B) started to loose stools of watery faeces indicative of the onset of parasitic gastroenteritis. In early September a few of them exhibited diarrhoea, while treated calves did not show overt symptoms of trichostrongyle infections. The calves in Group B were treated on 16 October, when the experiment was terminated.

Other recordings

The availability of grass on the pastures was high in the period May-July, presumably related to a rather high precipitation. However, scarcity of the grass became marked on both plots in the beginning of August, which was unusually dry in this particular year. Thus, the decrease in grass availability on the pastures was mostly due to low rainfall compared with the figures for an average year (the actual precipitation in August was 11.4 mm compared with the average value of 78 mm). For this reason the grazing area of both groups was extended to incorporate an additional ½ hectare of aftermath per group on 10 August (previously mentioned). However, the amount of grass was still insufficient due to low rainfall. To compensate for the low nutritional value of the diet, supplementary feeding with concentrate was introduced for both groups on 25 August. On an average each calf was given 1 kg of concentrate (grounded barley) per day.

Discussion

The present investigation on first season calves was designed to assess the effect of a treatment strategy consisting of 3 doses of ivermectin administered 3, 8 and 13 weeks after turnout on the build-up of infective larval loads on set-

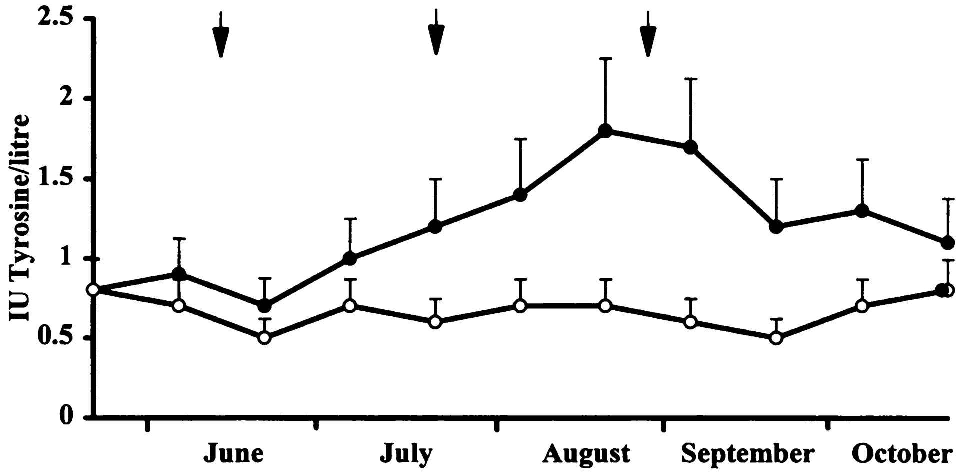

Figure 3. Mean serum pepsinogen levels ± standard deviation of two experimental groups of calves (see Fig. 2 for legend).

stocked pastures, and hence the risk of clinical and loss-producing trichostrongylosis later on. The larvae that were isolated from herbage at the start of the grazing season were obviously overwintered larvae derived from eggs excreted by calves the previous year. They did not have any significant impact on the health of the animals, but they established as adult worms in the non-treated calves and subsequently moderate numbers of eggs were passed by these animals, re-contaminating the pasture. A significant rise in the numbers of infective larvae on the grass was observed from the middle of July onwards, which is in agreement with results from other Northern European countries (*Bürger* 1966, *Michel* 1969, *Nilsson & Sorelius* 1973, *Henriksen et al.* 1976b, *Nansen* 1993). Peak larval counts were reached in the beginning of August.

The high pasture infectivity (group B) evidently led to increased parasitism as reflected in high egg counts by the end of August. This was accompanied by signs of clinical trichostrongylosis in most non-treated calves and was substantiated by elevated pepsinogen levels. The latter finding indicates that infection with *Ostertagia* spp. played a major role (*Jennings et al.* 1966). The beneficial effects of 3 consecutive ivermectin treatments were reflected in significantly reduced egg counts and pepsinogen levels in the treated Group A compared to the non-treated control Group B. This explains the higher weight gains of the treated calves in the second part of the grazing season, where these on an average gained 28 kg more than those in the non-treated group. The results of this experiment confirm previous studies (*Taylor et al.* 1985, *Armour et al.* 1987, *Ryan et al.* 1986, *Hollanders et al.* 1992, *Satrija et al.* 1996), which showed that strategical ivermectin treatments given to first-season grazing calves 3, 8 and 13 weeks after turnout effectively prevent build-up of high larval pasture loads from midsummer onwards. It is noteworthy that the efficacy of ivermectin apparently was lower against *Cooperia* spp. than against *Ostertagia* spp. Thus, results from faecal cultures showed that larvae of *Cooperia* spp. were cultured from fae-

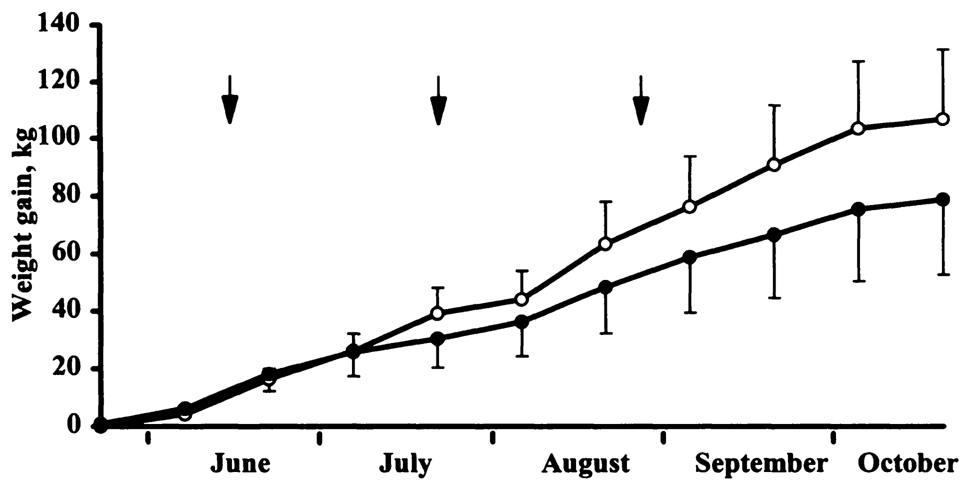

Figure 4. Cumulated average body weight gains ± standard deviation of the 2 experimental groups of calves (see Fig. 2 for legend).

ces obtained from treated calves throughout the grazing season. This finding is in accordance with observations by *Borgsteede & Hendriks* (1986) who showed that in mixed trichostrongyle infections, only larvae of *Cooperia* spp. could be cultured from faeces soon after treatment with ivermectin. Similarly *Steffan & Nansen* (1990) showed that egg excretion of *Cooperia* spp. was significantly suppressed only for 2 weeks after ivermectin treatment, whereas the suppressive effect against *Ostertagia* spp. was longer.

The overwintered herbage larval infectivity in this study was apparently not high enough to cause immediate clinical effect, as it was shown in an earlier study carried out in Lithuania by *Šarkūnas et al.* (1998). This presumably could be explained by delayed turnout of calves. According to old management traditions in many farms in Lithuania first season calves are not turned out until late May or beginning of June. This is in line with Danish observations (*Nansen et al.* 1987) which showed that a delayed turnout of calves of 3-4 weeks, i.e. in early June effectively reduced gastrointestinal parasitism.

In the present study it was shown that the seasonal fluctuation in herbage larval infectivity in Lithuania follows a pattern similar to Sweden (*Nilsson & Sorelius* 1973) Denmark (*Nansen* 1993), The Netherlands (*Jansen* 1977), and Norway (*Tharaldsen* 1976) i.e. an increase in herbage larval populations is observed in the middle of July as the result of recontamination of the pasture from susceptible animals that have picked up the overwintered trichostrongyle larvae in the beginning of the grazing season.

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Sammendrag

Strategiske ivermectin-behandlingers betydning for parasitisme hos kalve udsat for trichostrongylide infektioner på permanent græsmark i Litauen.

Undersøgelsen beskriver effekten af strategiske ivermectinbehandlinger mod trichostrongylider hos førstegangsgræssende kalve på naturligt smittede græsmarker. I alt 20 kalve blev fordelt på 2 hold efter vægt. Holdene blev udbundet den 22. maj på sammenlignelige forsøgsarealer, hver på 1 hektar. Hold A (mark A) blev behandlet med ivermectin 3, 8 og 13 uger efter udbindingen, mens hold B (mark B) fungerede som et ubehandlet kontrolhold. Efter første behandling faldt hold A's fækale ægudskillelse til lave værdier, som forblev lave gennem behandlingsperioden og sæsonen ud. Kontrolholdets ægudskillelse var indledningsvist moderat og steg til høje værdier. Græssets larveindhold på mark B (kontrol) steg til høje værdier i juli, i modsætning til larveindholdet på mark A (ivermectin), som forblev på lavt niveau hele sæsonen. De to hold havde sammenlignelig tilvækst frem til midten af juli, men fra dette tidspunkt voksede de behandlede kalve bedre end kontrollerne, som tillige viste kliniske tegn på parasitær gastroenteritis hen mod sæsonens slutning. Det konkluderes, at de strategiske ivermectinbehandlinger havde særdeles god effect. Fækal ægudskillelse, antal larver i græs samt serumpepsinogen niveauer viste, at denne effekt primært skyldtes en suppression af græskontamination.

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