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Targeted selective treatment of lungworm infection in an organic dairy herd in Sweden

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Abstract

The effect of targeted selective anthelmintic treatment on the seroprevalence of the lungworm *Dictyocaulus viviparus* in cattle was investigated. The study was commenced on an organic dairy enterprise in Sweden in November 1998 after the observation of an outbreak dictyocaulosis in the herd, and then continued for almost 3 years. The first year sampling was conducted on a monthly basis and then biannually with the exception of between August and November 2000 when sampling was performed monthly following a second outbreak of dictyocaulosis. Throughout the study, blood samples were examined for specific IgG_1 levels from all animals in the herd that had been grazing for more than 3 months. At the first sampling occasion, 13% out of the 90 blood samples were seropositive. One month later, after targeted selective treatment with eprinomectin (Eprinex®, Merial), the whole herd was seronegative. Seroprevalence then gradually increased and 1 year later it returned to levels similar to those observed at the start of the study. At turnout in April 2000, seroprevalence was 1.3% but it then rapidly increased to 28% and 30% in August and September, respectively. This increase was mainly due to an increase in FSG animals of which many were coughing. Consequently, all seropositive animals were injected with ivermectin (Ivomec®, Merial) at 0.05 mg/kg body weight in late August 2000. Although all animals recovered, seroprevalence was only reduced to 12% 1 month later. The differences in seroprevalence after both of these anthelmintic treatments were probably attributed to the timing. The first deworming with eprinomectin was conducted in November when the infection already was transient, whereas ivermectin in connection with the second outbreak was injected in a more acute phase of the infection cycle. Infection levels in 2001 were low with seroprevalences of 2.3% and 5.6% in May and September, respectively. These results show that dictyocaulosis in Sweden can be effectively controlled by the use of macrocyclic lactones. However, the infection was not eradicated from the herd despite close monitoring of the seroprevalence and targeted selective treatment of every seropositive animal on two occasions.
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Keywords: Cattle; Ruminant; Dictyocaulosis; Anthelmintic; Seroprevalence

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1. Introduction

Dictyocaulosis in cattle is caused by the extremely pathogenic lungworm *Dictyocaulus viviparus*. Infective third stage larvae (L3) are ingested with contaminated grass, penetrate the lymphatic system of the small intestine, enter the circulation and travel via the mesenteric lymph nodes to the lungs. Here the infection increases mucus production and induces a severe inflammatory response associated with emphysema and eosinophilia (Schnieder et al., 1991). This may cause lung congestion, which is associated with considerable socio-economic consequences as the performance of animals are retarded (Ploeger et al., 1990).

Although dictyocaulosis is a problem mainly of the non-immune first season grazers, all age classes of cattle may be infected (David, 1997; Höglund et al., 2001a). In a recent Swedish seroepidemiological field survey about 40% of 79 herds were found to be lungworm seropositive (Höglund et al., 2004b). This demonstrates that *D. viviparus* is fairly abundant in Sweden and that a significant part of the cattle population is affected like elsewhere in temperate regions of Europe where cattle graze on pasture (Ploeger et al., 2000; Schnieder et al., 1993). During the 1970s and 1980s efficacious broad-spectrum anthelmintics became available and many farmers now extensively use these drugs. From this it might be expected that dictyocaulosis would have become a disease of the past. However, during the 1990s, a dramatic increase in the number of lungworm outbreaks was recorded, especially in adult cows (Ploeger, 2002).

Also in Sweden, *D. viviparus* has received renewed attention. This has mainly been attributed to the increased interest for organic production (Höglund et al., 2001b). In contrast to other countries the irradiated larval lungworm vaccine has never been available on the Swedish market. Furthermore, there is no specific prophylactic programme advocated based on the application of anthelmintics. Although it has been shown that the risk for infection will be reduced if mixed or sequential grazing between first season grazers and cows and/or second season is avoided (Eysker et al., 1994b), it is difficult to apply this grazing strategy in Sweden. This is the case both in dairy herds where calves are born more or less all year around, and in beef herds with suckling calves. Instead whenever dictyocaulosis is diagnosed, Swedish farmers are advised to treat all animals in the affected grazing group, including those individuals harbouring subtle sub-clinical infections. There is information on the prevalence of *D. viviparus* in various regions, and how this parasite responds to anthelmintic treatment (see for example, Borgsteede et al., 1988; Molento et al., 1999; Ploeger et al., 2000; Schnieder et al., 1993). However, knowledge about the long-term seasonal dynamics of the infection and how this is influenced by deworming under natural conditions in Sweden is missing.

This paper reports on naturally acquired *D. viviparus* infection in an organic dairy enterprise in central Sweden. The objective was to monitor the long-term dynamics of lungworm infection in connection with selective targeted anthelmintic intervention following an outbreak of clinical disease. Variations in seroprevalence in the herd were monitored on a more or less regular basis for almost 3 years. By this approach it was also possible to validate the diagnostic value of the lungworm Ceditest ELISA under practical field conditions.

2. Material and methods

2.1. Animals and study site

The study was conducted between November 1999 and September 2001 on a commercial organic dairy enterprise situated 30 km east of Uppsala in central Sweden. It was initiated following a massive outbreak of dictyocaulosis in the herd, which resulted in the death of one heifer. This animal was sent in for post-mortem examination at the National Veterinary Institute in Uppsala, Sweden. Although no adult worms were found, it was confirmed that this animal had died from lungworms as the lungs showed the characteristic pathological lesions and because it was seropositive (24%) according to the Ceditest ELISA (Cornelissen et al., 1997). The farmer was informed and it was subsequently formally agreed that animals in the herd should be closely monitored.

Altogether 1559 blood samples from 175 animals from: (1) lactating cows, (2) heifers and (3) first season

grazers (FSG), were collected. The cows were stabled each year between the end of September until late April or early May, whereas FSG > 8 weeks old and most of the heifers were housed on a straw bed in a roofed shed in pens of approximately 10 animals. Although age segregated grazing was applied on two nearby situated 1.2 ha pastures, there was a continuous flow of calves into the group of FSG and heifers, at the same time as heifers were regularly introduced into the cow group prior to their first lactation. In 1999, some heifers were grazed for a 3-week period on a natural pasture situated approximately 5 km away from the rest of the herd.

As this study was conducted on an organic farm, animals could only be dewormed selectively following diagnosis. The first intervention was in November 1998 with eprinomectin (Eprinex®, Merial), administered topically at the normal dose rate of 0.5 mg/kg bodyweight, whereas the second was in September 2000 with ivermectin (Ivomec®, Merial), injected subcutaneously at the recommended dose rate of 0.05 mg/kg body weight. During the first outbreak only seropositive animals were treated, whereas following the second outbreak all seropositive adult cattle and all first season grazers were treated.

2.2. Sampling

Blood sampling was conducted at monthly intervals between November 1998 and October 1999, and between August and November 2000. Then sampling was performed biannually, before turnout in late April or early May, and at housing in September. Collection of faeces was only performed at the first sampling occasion followed by examination for first stage larvae with the Baermann method based on 30 g of faeces. At all other sampling occasions, blood samples were analysed from every individual in the herd that had been out grazing for a minimum of 3 months. The serological method used was the Ceditest, ID-DLO, Lelystad, The Netherlands, as described in detail elsewhere (Cornelissen et al., 1997). Briefly, this is an enzyme-linked immunosorbent assay (ELISA) technique that is specific against patent lungworm infections. With this test an animal is considered as infected when the seropositivity is ≥15% in relation to control sera added on each ELISA plate.

2.3. Tracer test

In 2001 the infective larval pick-up from pasture used by the first-season grazers and heifers was estimated by the use of two sequential groups of six parasite-free naive tracer calves. The groups were sequentially introduced on the pasture for periods of 9 and 12 weeks, respectively. The first group was allowed to graze from turn out in mid May until mid July, whereas the second group were out during the second part of the grazing period from mid July until mid September. Both groups were housed for additional 4 weeks before they were sacrificed and the number of established worms was determined after perfusion of the lungs according to procedures described by (Borgsteede et al., 1998).

3. Results

3.1. Animals

A total of 1559 blood samples from 173 animals were examined with a monthly average 82 ± 8 animals per month. Out of the 173 animals, 81 (46%) were replaced during the study. Only 25 (14%) of the animals were examined for all 19 sampling occasions demonstrating the rapid turnover in this herd. Fifty calves were recruited from cows calving the year around, whereas 27 animals of various ages were brought in from other herds on three separate occasions. This created a situation with a continuous inflow of susceptible animals into the herd.

3.2. Variations in seroprevalence

Infection levels varied considerably during the course of the study, both within and between years (Fig. 1). In November 1998, when the study was initiated, 12 (13%) out of 90 animals in the herd were seropositive and out of these 7 (58%) also shedded an average of 23.5 ± 17 *D. viviparus* infective third stage larvae. However, 1 month later, following selective targeted eprinomectin treatment of all animals that were seropositive the previous month, the infection level was reduced to 0%. Thereafter seroprevalence gradually increased and

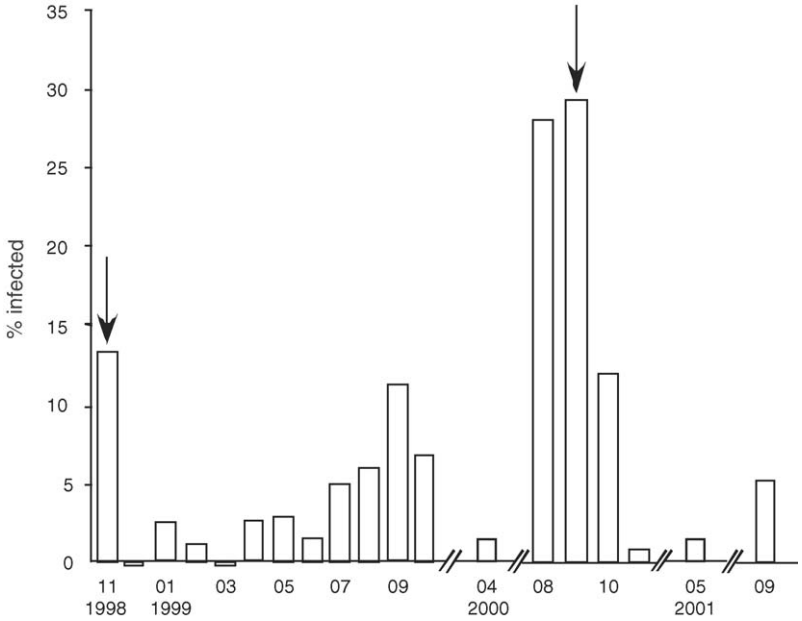

Fig. 1. Variations in the seroprevalence of *Dictyocaulus viviparus* in an organic dairy herd in Sweden between November 1998 and September 2001. Results are based on the Ceditest ELISA in which animals with a seropositivity ≥15% are considered infected. Arrows indicate the timing of selective targeted anthelmintic intervention of all seropositive animals in the herd. The first deworming was with eprinomectin (Eprinex®, Merial), whereas the second with ivermectin (Ivomec®, Merial) at the recommended dose rates, $n = 67–90$.

in September 1999, it was back to almost the same level as observed the previous year. At this time there were no signs of clinical disease and all animals were left untreated.

Following the stable period prior turnout in April 2000, seroprevalence was 1.3%. However, infection levels increased dramatically during the grazing season 28% and 30% of the animals in the herd were seropositive in August and September 2000, respectively. The great majority of these animals had dictyocaulosis and were therefore injected with ivermectin at the recommended dose rate. However, 1 month after deworming, seropositivity was reduced to 13% in the herd, whereas 1.0% of the animals were seropositive in November 2000. At turnout the following year in May 2001, seroprevalence was still low with 2.3% of the animals in the herd infected. In contrast to previous years, a slight increase was observed after the grazing period, with 5.6% seropositive animals in September 2001.

Most individuals seroconverted early in life and were normally seropositive for some months. However, certain individuals were seropositive, or close to, on repeated occasions. In some instances they remained so throughout the study period (see for example 489, 779, 809 and 823 in Fig. 2). Two such animals (489 and 890) were actually dewormed both in November 1998 and in August 2000.

3.3. Tracer test

The results of the Ceditest ELISA and the number of animals shedding larvae are shown in Fig. 3. Optical densities above the cut-off value were only recorded in two animals in the second grazing group. Although these animals excreted low numbers of larvae, these were only observed on a few occasions. Low numbers of adults were recovered at slaughter from some animals in both grazing groups. In the first grazing group four animals were infected, but only with an average of 3.3 adult worms. Thus, the use of tracer animals confirmed the presence of infective larvae on the pasture, although the pasture contamination was minimal during the summer months of 2001.

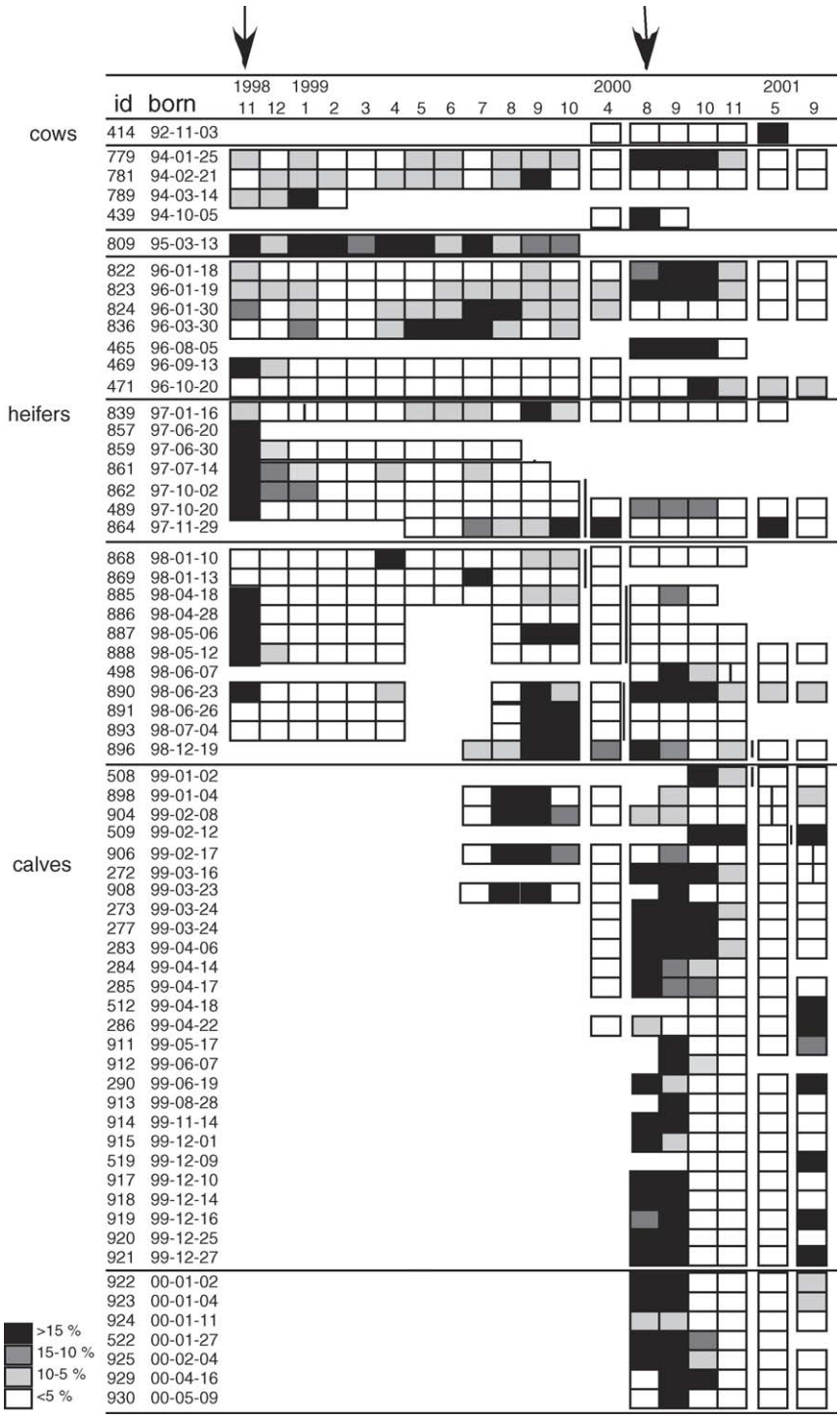

Fig. 2. Individual variations in seroprevalence in animals with a seropositivty ≥15% at any of the 19 sampling occasions. id = animal identity, born = date of birth. Shaded blocks indicate the level of seropositivity, whereas vertical bars indicate the date for the first lactation.

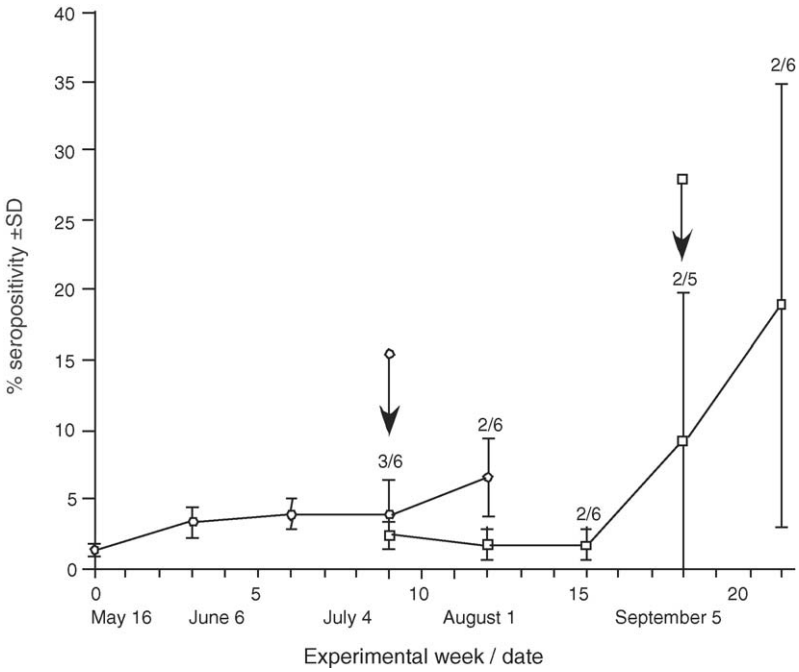

Fig. 3. Results from a tracer test performed with lungworm naïve first season grazing calves during the grazing period of 2001. Circles denote the mean values for the first grazing group, whereas the boxes show the values for the second grazing group. Both groups were composed of six calves. Figures in the graph indicate the number of animals that were shedding larvae, whereas arrows indicate when the animals were housed.

4. Discussion

In this study seroprevalence of *D. viviparus* in cattle fluctuated both within and between years in a herd on an organic dairy farm in central Sweden. Although selective targeted anthelmintic treatments following two outbreaks of dictyocaulosis proved to be successful, seropositivity was not reduced in a similar fashion following each of these outbreaks. Infection levels were dramatically reduced, especially after the second outbreak, but eradication of the infection from the farm was unsuccessful. There are several possible reasons for this. First, it can be argued that the serological test used in the present study (Ceditest) may lack the sensitivity to detect all infected animals. To start with, it is based on an adult worm antigen (Cornelissen et al., 1997). Furthermore, animals vaccinated with irradiated larvae do not result in measurable antibody titres (de Leeuw and Cornelissen, 1991). Therefore it is unlikely that larval populations will be detected. Secondly, the Ceditest may fail to detect animals with a very low adult infection. In fact this is also shown in the results from the current tracer test, where small numbers of worms were found in seronegative calves. Unfortunately, irrespective of whether these factors act alone or in combination, lack of sensitivity is a common problem of any of the currently employed non-destructive diagnostic tests used to detect lungworm infection in cattle.

Traditionally, diagnosis of lungworms in cattle is confirmed by demonstration of first stage larvae in faeces by the Baermann method. Although it is possible to detect animals with very few worms using this method (Eysker, 1997), animals solely with larval infection cannot be diagnosed, by definition. From the mid 1980s, however, various serological tests became available (Bos and Beekman, 1985; Cornelissen et al., 1997; Tenter et al., 1993). In the present study antibody levels were determined with ELISA, by which it has been shown that it takes approximately 4–5 weeks before seroconversion can be detected in experimentally infected calves (Cornelissen et al., 1997). In contrast, when animals were boosted with L3s 10 weeks apart, they seroconverted after 2 weeks (Höglund et al.,

2003). In the current study, the animals were naturally infected on pasture. Accordingly, they were gradually exposed to low levels of infective larvae, as was also indicated by results from the tracer test performed in 2001. Whenever possible, the animals in the herd were re-examined and in a few instances up to 19 times. As demonstrated in Fig. 2, some individual animals remained seropositive, or near to, on repeat occasions. Taken together, these data show that the ELISA employed in the present study produced consistent and reliable results. However, as indicated by the tracer test, low levels of infection may be missed with the recommended cut-off value of 15% seropositivity. Still, antibody levels increased somewhat towards the end of the grazing periods in both tracer groups.

Due to logistic reasons faecal examination was only performed on the first sampling occasion. Then it was demonstrated that the results based on serology largely agreed with those obtained with faecal examination. This is in accordance with a study of lungworms in adult dairy cattle (Borgsteede et al., 2000). As indicated previously (Höglund et al., 2003), a major shortcoming of faecal examination is that larval shedding is restricted to a limited period of some weeks, whereas antibodies remain in the blood for several months. This is an advantage at least when the purpose of the diagnostic investigation is to confirm the presence of lungworms in the herd. It was also highlighted in the present study that sampling of blood in a field situation is less time consuming than faecal examination. Thus, for surveillance of lungworm infection it seems preferable to focus on serology, in particular when sampling is conducted around housing.

Although, knowledge about the half-life of anti-lungworm antibodies is limited, it has been shown that it takes some months for these to decay (Höglund et al., 2004b). It is reasonable to assume that the time course for this is dependent on the original antibody levels, as was also indicated by the results of the present study. Still, it is obscure as to why all animals at the second sampling occasion were already seronegative 1 month after eprinomectin treatment, whereas some individuals remained seropositive up to 2 months after ivermectin injection, especially since both compounds are known to be highly effective against *D. viviparus* (Benz and Ernst, 1981; Cramer et al., 2000). The most reasonable explanation is that eprinomectin in connection with the outbreak in 1998 was administered at a time point when the infection already was transient, whereas ivermectin treatment in 2000 was injected earlier in the year and thus in a more acute phase of the outbreak.

It is well known that macrocyclic lactones are effective against *D. vivparus*, and that animals may stay in the same pasture provided that they are treated at regular intervals (Armour et al., 1987; Lyons et al., 1981). Pour-on formulations are convenient, and in particular those with eprinomectin that can be used without problems in lactating animals as there is no milk withdrawal period (Shoop et al., 1996). So far there are no published reports on, or evidence for, anthelmintic resistance against lungworms in cattle. According to the present results however, eprinomectin was not fully effective against all parasitic stages of *D. viviparous*, as certain individuals (809 and 789 in Fig. 2) seroconverted and became positive again soon after treatment. This was despite the fact that they were dewormed during the housing period. Similar results have been obtained with abamectin (avermectin B1), which was found to be only 93.8% effective against *D. viviparus* (Williams et al., 1992). However, in the present study drug failure could equally be due to poor uptake of the drug and thus a reduced bioavailability of the active substance. Alternatively, these animals may have been re-infected during the housing period. The possibility of pick-up of larvae in housed cattle has been described and it cannot be ignored (Grønvold and Jørgensen, 1987). However, this is a doubtful explanation in the current study, as the few animals that were seropositive on repeated occasions during housing, or slightly below the threshold, were exclusively older animals showing no signs of disease. Accordingly, these individuals were more likely silent carriers that had been infected earlier on pasture.

Similar to other pasture borne parasites, lungworm larvae develop and survive when the conditions are warm and moist on pasture (Duncan et al., 1979). Elsewhere in Europe it has been shown that infective L3 stages may overwinter on pasture (Oakley, 1982). This was also indicated by the results in the present study, and confirms the view that survival on pasture may occur sporadically in Sweden (Höglund et al., 2001b). However, as indicated above, some animals in the present study were seropositive, or near to, more or less throughout the study, but without showing the typical clinical signs of dictyocaulosis. Although it is

unlikely that animals with exclusively inhibited populations will be tested positive with the Ceditest (de Leeuw and Cornelissen, 1991), it was indicated that certain individuals served as long-term carriers of the infection. This is in agreement with studies both from the UK and The Netherlands where it has been shown that a higher proportion of cows or yearlings shed larvae in spring than in winter (Eysker et al., 1994a; Oakley, 1977; Saatkamp et al., 1994). Taken together, inhibited development is probably the most important strategy of lungworm to overwinter also in Sweden. Seropositive animals in this study could broadly be divided into high and low responders. The reason why some animals developed immunity and others not is obscure, and needs to be addressed in future studies.

Furthermore, it has been speculated as to whether there is a spread of lungworms between wild ruminants and domestic cattle. Although it has been shown that calves may be infected when inoculated with reciprocal larvae of closely related species of lungworms, larval establishment is generally poor (Bienioschek et al., 1996). Recently conducted studies have also shown that there is no such transmission in Sweden, despite the fact that lungworm-infected roe deer and moose are rather prevalent in areas where cattle are grazing (Divina et al., 2000).

The animals that were introduced from other farms in the present study were not treated before turnout. Thus the introduction of lungworm with these animals cannot be ruled out. On the other hand, none of the farms where these animals came from had any recent history of dictyocaulosis and most animals never had access to pasture on the original farm. This in itself implies that the likelihood for an introduced infection with these animals was minimal. Recently conduced studies on *D. viviparus* have shown that lungworm populations in Sweden are structured genetically and form distinct subpopulation on different farms (Hu et al., 2002; Höglund et al., 2004a). This view was supported by the current results showing that the infection was persistent and that seropositive animals were present in the herd almost throughout the whole study period, although sometimes at very low levels. Anyhow, both outbreaks of dictyocaulosis were sporadic and they were also observed late during the grazing season from August and onwards, which is in agreement with earlier studies (Schnieder et al., 1993). Still the outbreak in August 2000 was unexpected even though seroprevalence was monitored on a regular basis. This shows that infection levels may build up to pathogenic levels very quickly.

In conclusion, this study has provided important general information about the seroepidemiology of lungworm infection in a commercial dairy herd in Sweden. In particular, it has improved our knowledge about how infection levels are related to anthelmintic intervention in connection with outbreaks of clinical disease. It was also demonstrated that it is possible to control dictyocaulosis by the use of macrocyclic lactones, even though the animals were not dewormed until clinical signs of disease were observed. However, eradication of the infection from the herd was unsuccessful despite close surveillance and targeted selective treatment of all seropositive animals on two different occasions 2 years apart. Although the reasons for this are obscure, it may be due to lack of sensitivity of the Ceditest in combination with re-infection of susceptible animals by pick-up of larvae from pasture. This study highlights both the importance of certain individuals that act as seasonal carriers of the infection, and occurrence of pick-up of low levels of infection throughout the entire grazing season. However, signs of disease were only observed later than early autumn. Taken together it seems difficult to eradicate lungworms in Sweden despite intensive monitoring and selective targeted treatment of every seropositive animal on repeated occasions.

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