Prevalence and Intensity of Infection of *Cryptosporidium* spp. and *Giardia duodenalis* in Dairy Cattle in Galicia (NW Spain)

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With 1 figure and 1 table

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Summary

Faecal samples were collected from 734 cattle selected at random from 60 dairy farms in Galicia (NW Spain). The animals studied were classified into 12 age groups: <1 month (53); 1–5 months (30); 6–11 months (31); 12–16 months (72); 17–20 months (64); 21–24 months (96); 3 years (94); 4 years (74); 5 years (67); 6 years (67); 7–8 years (63) and 9–13 years (23). Oocysts of *Cryptosporidium* spp. were identified in 104 animals (14.2%) distributed throughout all of the age groups and from 40 different farms (66.7%). The percentage of cattle infected ranged between 58.5% in calves <1 month and 7.9% in 13 years old in Galicia, the major dairy cattle farming region in Spain.

Materials and Methods

The study was carried out in Galicia (NW Spain), a region characterized by a high density of dairy cattle farms. Between January and June 2005, faecal samples were collected from 734 cattle selected at random from 60 dairy farms. The animals studied were classified into 12 age groups: <1 month (53); 1–5 months (30); 6–11 months (31); 12–16 months (72); 17–20 months (64); 21–24 months (96); 3 years (94); 4 years (74); 5 years (67); 6 years (67); 7–8 years (63) and 9–13 years (23). The faeces were classified according to their consistency as diarrhoeic or non-diarrhoeic. Identification of *Cryptosporidium* spp. oocysts in faecal samples from calves <1 month was carried out using the Heine’s (1982) method of direct examination. The intensity of infection was evaluated according to Castro-Hermida et al. (2002b).

Potential reductions of both diseases can potentially reduce the growth of ruminants (Starkey et al., 2005). Adult cattle are generally considered refractory to heavy infections by *Cryptosporidium* spp. or *G. duodenalis* and associated clinical diseases because of a strong immune response. Nevertheless, it has been shown that asymptomatic adult cattle may act as healthy carriers and may be a source of infection for younger animals, especially during the periparturient period (Fayer et al., 2000a; Ralston et al., 2003). Moreover, several researchers and public health officers have concluded that cattle play a major role in the contamination by both of the parasites of surface water used for drinking and recreational purposes. The objective of the present study was to determine the prevalence and intensity of infection of *Cryptosporidium* spp. and *G. duodenalis* in dairy cattle between <1 month and 13 years old in Galicia, the major dairy cattle farming region in Spain.
intensity of infection in the different age groups were compared by a test of comparison of proportions and the Kruskal–Wallis test (non-parametric ANOVA), respectively, using the GraphPad InStat® (version 3.05; GraphPad Software, San Diego, CA, USA). The intensity of infection by Cryptosporidium spp. in calves <1 month was not evaluated as the number of oocysts per gram of faeces, therefore, these data were not included in the statistical analysis.

Results

Oocysts of Cryptosporidium spp. were identified in 104 animals (14.2%) distributed throughout all of the age groups and from 40 different farms (66.7%). The percentage of cattle infected ranged between 58.5% in calves <1 month and 7.9% in 7- to 8-year-old cows, i.e. the percentage of infection decreased significantly (P < 0.05) with increasing age. Cysts of G. duodenalis were identified in 221 animals (30.1%) from 56 farms (93.3%). The parasite was detected in all age groups, at rates of infection ranging between 21.8% (9–13 years) and 56.7% (1–5 months), although these differences were not statistically significant. Concurrent infections were observed in all groups, except those comprising 3-year-old animals. The highest percentages of animals infected by both parasites occurred in the groups of calves of 1–5 months (23.3%) and 6–11 months (25.8%). The prevalence of infection in the different age groups is shown in Fig. 1. For Cryptosporidium spp. the intensity of infection in animals older than 1 month ranged between 10 and 5924 oocysts/g of faeces and there were no significant differences between the different groups. The mean intensity of infection by G. duodenalis ranged between 7 and 15 412 cysts/g of faeces, with the number of cysts shed being significantly higher (P < 0.05) in calves <1 month than in calves aged 1–5 months. The mean intensity of infection by Cryptosporidium spp. and G. duodenalis in the different age groups is shown in Table 1. Symptoms of diarrhoea were only found in calves aged <1 month and concur- rent infections were observed in all groups, except those comprising 3-year-old animals. The highest percentages of animals infected by both parasites occurred in the groups of calves of 1–5 months (23.3%) and 6–11 months (25.8%). The prevalence of infection in the different age groups is shown in Fig. 1. For Cryptosporidium spp. the intensity of infection in animals older than 1 month ranged between 10 and 5924 oocysts/g of faeces and there were no significant differences between the different groups. The mean intensity of infection by G. duodenalis ranged between 7 and 15 412 cysts/g of faeces, with the number of cysts shed being significantly higher (P < 0.05) in calves <1 month than in calves aged 1–5 months. The mean intensity of infection by Cryptosporidium spp. and G. duodenalis in the different age groups is shown in Table 1. Symptoms of diarrhoea were observed in 64 animals (8.4%) belonging to all age groups, but significant associations between parasitisation and the consistency of the faeces were only found in calves aged <1 month infected by Cryptosporidium spp. and 1–5-month-old animals infected by G. duodenalis.

Discussion

This study is the first extensive and quantitative investigation of G. duodenalis cyst shedding in the faeces of apparently healthy young and adult dairy cattle in Galicia. Cryptosporidium spp. oocyst excretion in the faeces of adult cattle has not been evaluated either in this region. Prevalence rates and percentage of farms testing positive show that cryptosporidiosis and giardiosis are widespread in dairy cattle in Galicia. It is difficult to compare the present data with that reported in other similar studies because in the latter, animals of different ages were examined or the farms were chosen because of a history of diarrhoea, or because animals displayed symptoms of enteric illness. However, we believe that the mean prevalence per farm found in the present study (66.7% for Cryptosporidium spp. and 93.3% for G. duodenalis) reflects a serious situation, taking into account that the study was carried out on apparently healthy adult animals, the farms were selected at random and the only possible restriction was whether the farmers consented to the study. The actual prevalence of infection may even be underestimated because only one faecal specimen was collected per animal. If that specimen was identified as negative during a period when the animal was excreting oocysts intermittently, then the animal would be considered negative. Cumulative prevalence of 100% has been reported for both parasites in dairy calves at specific locations (O’Handley et al., 1999; Castro-Hermida et al., 2002b). However, in most cross-sectional studies, the reported infection rate has been lower (Fayer et al., 2000b; Wade et al., 2002). Ingestion of oocysts had been reported for both parasites in dairy calves at specific locations (O’Handley et al., 1999; Castro-Hermida et al., 2002b). However, in most cross-sectional studies, the reported infection rate has been lower (Fayer et al., 2000b; Wade et al., 2002; Castro-Hermida et al., 2002a). G. duodenalis was more prevalent than Cryptosporidium spp. in all groups except in calves <1 month. In the present study, Cryptosporidium spp. was most prevalent in animals <1 month of age, with prevalence decreasing sharply afterwards; the highest prevalence of G. duodenalis was observed in calves of 1–5 months of age. These results are therefore consistent with those of studies in North America and Europe where similar levels of infection have been reported both in

### Table 1. Intensity of Cryptosporidium spp. oocyst and G. duodenalis cyst shedding in dairy cattle in relation to age

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Oocysts per gram of faeces (mean ± SD)</th>
<th>Cysts per gram of faeces (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 month</td>
<td>ND*</td>
<td>1338.3 ± 1769.1</td>
</tr>
<tr>
<td>1–5 months</td>
<td>181.1 ± 251.4</td>
<td>2015.8 ± 4469.2</td>
</tr>
<tr>
<td>6–11 months</td>
<td>52.1 ± 36.5</td>
<td>154.5 ± 303.7</td>
</tr>
<tr>
<td>12–16 months</td>
<td>50.2 ± 47.6</td>
<td>96.9 ± 100.5</td>
</tr>
<tr>
<td>17–20 months</td>
<td>60.7 ± 44.4</td>
<td>142.4 ± 410.9</td>
</tr>
<tr>
<td>21–24 months</td>
<td>83.2 ± 98.8</td>
<td>62.8 ± 74.9</td>
</tr>
<tr>
<td>3 years</td>
<td>25.5 ± 11.7</td>
<td>83.2 ± 207.7</td>
</tr>
<tr>
<td>4 years</td>
<td>25.8 ± 26.1</td>
<td>40.5 ± 27.6</td>
</tr>
<tr>
<td>5 years</td>
<td>131.0 ± 144.9</td>
<td>59.8 ± 38.7</td>
</tr>
<tr>
<td>6 years</td>
<td>50.5 ± 43.2</td>
<td>73.6 ± 52.5</td>
</tr>
<tr>
<td>7–8 years</td>
<td>1225.6 ± 2626.6</td>
<td>114.0 ± 183.2</td>
</tr>
<tr>
<td>9–13 years</td>
<td>238.0 ± 114.5</td>
<td>116.0 ± 105.1</td>
</tr>
</tbody>
</table>

*No. of oocysts per gram of faeces was not determined.

The intensity of infection in this group (2.0 ± 1.1) was evaluated semiquantitatively according to the average number of oocysts in 50 randomly selected fields at 1000x magnification (0: absence of oocysts; 1: < 50 oocyst/field; 2: 5–50 oocysts/field; 3: 50–250 oocysts/field and 4: > 250 oocysts/field).

![Fig. 1. Prevalence of Cryptosporidium spp., G. duodenalis and concurrent (Cryptosporidium + G. duodenalis) infections in cattle aged from <1 month to 13 years.](image-url)
dairy and beef cattle. Calves usually became infected with *Cryptosporidium* spp. at between 1 and 4 weeks of age and shed oocysts for only about 10 days (Santín et al., 2004), although some authors have observed a second peak of prevalence in calves of approximately 6 months old (Huetink et al., 2001). Other authors have also observed a significant reduction in the prevalence of infection in relation to age (Sischo et al., 2000).

As regards *G. duodenalis*, the present results are consistent with those of O’Handley et al. (1999); Huetink et al. (2001) who observed the highest levels of cyst excretion in calves between 3–5 months of age. First excretion begins at 4 weeks with shedding continuing for over 6–8 months (Becher et al., 2004).

The mean intensity of infection by *Cryptosporidium* spp. and *G. duodenalis* in the different groups of age ranged between 25 and 1225 oocysts/g and 40 and 2015 cysts/g of faeces, respectively, figures that are similar to those of previous studies of apparently healthy cattle (Ralston et al., 2003).

We can conclude that asymptomatic adult dairy cattle are a real source of environmental contamination by *Cryptosporidium* spp. and *G. duodenalis* in Galicia, as indicated by the high prevalence of infection observed in the present study, and also because of the large number of dairy farms, the large proportion of adult animals (around 90%) in dairy herds and the large volume of faeces that they produce. The results of the present study demonstrated the importance of adult cattle in maintaining infection in dairy farms. However we cannot evaluate the real risk of human infection because we did not identify the species or genotypes of the infective agents.

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**References**


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