INTRODUCTION

Numerous species of mammals have been reported to be hosts of Cryptosporidium spp., intestinal coccidian parasites which are a common cause of diarrheal illness in man (including those immunosuppressed or infected with AIDS) and animals [8, 12, 14, 21]. A variety of domestic animals, including calves, appear to be important zoonotic reservoirs for C. parvum infection in man, while wild animals may also be a source of infection [4, 5, 6, 18, 20]. Since the early 1970s, cryptosporidial infection has been recognised all over the world as a cause of diarrhoea in neonatal animals, particularly calves, [1, 13, 15, 19]. Recently, it has been estimated that over 90% of the dairy cattle herds in the United States are infected with C. parvum [22] and in two different parts of Europe (Poland and Portugal), about 40% of calves with diarrhoea were found to be infected [10, 16].

Because of the world-wide importance of both cryptosporidiosis and giardiosis in humans and animals, and the absence of comprehensive data on the occurrence of these parasites in calves from Poland, the present study was conducted to determine the prevalence of infections in calves from arbitrarily selected farms of Wielkopolska macroregion. Additionally, the infectivity of C. parvum oocytes isolated from faeces of naturally infected calves was tested by the experimental transmission to mice.

MATERIALS AND METHODS

Specimen preparation and examination of faecal samples. During February to April 1997, faecal specimens
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were collected from 75 calves, aged from 3–16 days, from 9 farms in Wielkopolska region. Faecal smears prepared on glass slides were air dried, fixed in 100% methanol and stained using a modified Ziehl-Neelsen technique [9]. All slides were studied for typical acid-fast cryptosporidial oocysts at 400 × magnification. Specimens containing oocysts were categorised into three groups as follows: (+) < 5 oocysts, (++) 5 to 10 oocysts, (+++) > 10 oocysts in each of 20 microscopic fields.

Thirty five samples from 5 selected farms were examined by a direct immunofluorescent technique, the MerIFluor Cryptosporidium/Giardia assay as described by Bajer et al. [3]. The oocysts of C. parvum and cysts of Giardia sp. were measured using a calibrated eyepiece graticule.

Infection of laboratory mice with C. parvum. Isolates of C. parvum oocysts obtained from naturally infected calves were tested for their infectivity to mice. The oocysts were isolated from faeces mixed in 2.5% w/v K₂Cr₂O₇, passed through wire mesh sieves and stored at 4°C for no more than 1 month prior to use. The oocysts were purified and concentrated by flotation on sucrose gradients with two specific gravities of 1.103 and 1.064 respectively [2]. The oocysts were counted in 0.02 ml volume and if necessary, the number of oocysts for inoculation was determined by adjusting the volume. Before inoculation, oocysts were incubated for 24 hrs at 37°C with antibiotics (200 µg/ml of penicillin G and 100 µg/ml of gentamycin sulfate) and subsequently washed in PBS.

Ten inbred female BALB/c mice were orally inoculated at 4 weeks of age with 10⁴ oocysts each. Before inoculation, the mice were immunosuppressed using dexamethasone administered at a dose of 0.125 mg/mice/day in drinking water for 8 days. The patency of C. parvum infection under experimental conditions was confirmed by examination of faeces collected daily until the 7th day post inoculation (dpi). Endogenous stages of C. parvum were also sought in intestinal specimens of each inoculated mouse examined at necropsy on 7 dpi. Approximately 1 cm pieces of gut from the posterior jejunum and ileum were processed for histological examination after fixation in 10% buffered formal saline (pH 7.2), embedding in paraffin, sectioning at 5 µm thickness and staining with haematoxylin and eosin.

**RESULTS**

**Prevalence and intensity of infections with C. parvum and Giardia sp. in calves.** In the Wielkopolska macroregion oocysts of C. parvum were found on 6 (67%) of the 9 farms (Tab. 1). The prevalence of infections ranged from 20–88%, and intensity of infections were heavier on farms A, B and C. Using the MerIFluor test, oocysts of C. parvum were detected in 18/35 (51%) calves and cysts of Giardia sp. in 5/35 (14%) (Tab. 2). C. parvum oocysts were round, measuring approximately 4–6 µm, while Giardia sp. cysts were oval, measuring approximately 11–15 µm. Only one calf from farm D had a

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of calves examined</th>
<th>Number of positive(%)</th>
<th>Number of calves shedding oocysts with intensity of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>7 (88)</td>
<td>(+) 2</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>18 (72)</td>
<td>(++) 5</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>5 (71)</td>
<td>(+++) &gt; 10</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>6 (60)</td>
<td>(++) 5</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>2 (40)</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>1 (20)</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>38 (51)</td>
<td>13 12 14</td>
</tr>
</tbody>
</table>

(+) < 5 oocysts in each of 20 fields; (++) = 5 to 10 oocysts in each of 20 fields; (+++) > 10 oocysts in each of 20 microscopic fields, 400 × magnification.

**Table 1.** Prevalence of Cryptosporidium parvum infection in calves on some farms from Wielkopolska macroregion.

<table>
<thead>
<tr>
<th>Faecal specimens (positive (%))</th>
<th>Farm</th>
<th>Number of calves examined</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>10</td>
<td>7</td>
<td>1*</td>
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<tr>
<td></td>
<td>E</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>18 (51)</td>
<td>5 (14)</td>
<td></td>
</tr>
</tbody>
</table>

* coinfection of Cryptosporidium parvum and Giardia sp.

**Table 2.** Cryptosporidium parvum oocysts and Giardia sp. cysts detected by direct immunofluorescent test in calves from selected farms.

Figure 1. Oocysts of Cryptosporidium parvum (A) and cyst of Giardia sp. (B) stained with MerIFluor (Cryptosporidium/Giardia) direct fluorescence.
mixed infection of *C. parvum* and *Giardia* sp. Figure 1 shows oocysts of *C. parvum* and cyst of *Giardia* sp. stained with MerIFluor (*Cryptosporidium/Giardia*) direct fluorescence.

**Infection of BALB/c mice with *C. parvum* isolated from calves.** Oocysts of *C. parvum* were detected in the faeces of all experimentally infected mice from 2–7 dpi. Histological examination of the gut sections on 7 dpi revealed endogenous stages of *C. parvum* in all inoculated mice (Fig. 2).

**DISCUSSION**

The detection of both cryptosporidial oocysts and *Giardia* sp. cysts using direct immunofluorescent (MerIFluor *Cryptosporidium/Giardia*) test creates very sensitive and specific diagnosis of infection with these parasites.

The high prevalence and intensity of *C. parvum* infections in calves on some farms in the Wielkopolska macroregion indicates that they are common parasites in this region. In contrast, the low prevalence and intensity of *Giardia* sp. infection on farms indicates they are rare in this region. Calves infection with *C. parvum* in some studied farms creates a condition for zoonotic transmission and environmental spreading of oocysts through direct or soil-water routes.

Experimental infections showed that *C. parvum* isolates from naturally infected calves were infective to immunosuppressed laboratory mice. According to Current and Blagburn [6], adult laboratory mice cannot be readily infected with *C. parvum*, whereas neonates (1-day old mice) orally inoculated with *Cryptosporidium* oocysts of calf origin were heavily infected [16]. It seems more likely that *C. parvum* from calves may be infective to wild rodents and could contribute to zoonotic reservoirs of infection.

However, controlling the spread of *C. parvum* or *Giardia* sp. requires the reduction or elimination of parasites from the environment, but this seems extremely difficult because oocysts and cysts are very resistant to various commonly used disinfectants [8]. Under favourable conditions they remain infectious for a relatively long time. Thus, not only immunosuppressed persons [10] but also immunocompetent farmers, veterinary, medical and laboratory workers are at high risk of infections associated with ingesting of cysts or oocysts from the environment contaminated with faeces of infected calves. Infections with both parasites in immunocompetent persons could be serious, but are generally self-limited and leave the host solidly immune to reinfection.

Very high prevalence and intensive shedding of cryptosporidial oocysts by calves on some farms presented in this study may indicate that naturally infected calves pose an essential source of *C. parvum* infections enabling wide environmental spreading of oocysts through direct or soil-water routes into wild animals and humans.

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**REFERENCES**


