**INTRODUCTION**

*Cryptosporidium* is an ubiquitous parasite which can cause acute and chronic infections of the gastrointestinal tract in humans and animals. The infective form is the oocyst excreted in the faeces. The species *C. parvum* is of particular importance to humans, because it can cause life-threatening diarrhea in immunocompromised individuals (Current, 1988). The significance of *Cryptosporidium* spp. as an aetiological agent of waterborne diseases in the USA and European countries has increased with the more widespread use of surface water for drinking water production (Ongerth & Stibbs, 1987; Rose et al., 1988; Hayes et al., 1989; Smith & Rose, 1990; Richardson et al., 1991; LeChevallier et al., 1991; Hansen & Ongerth, 1991; MacKenzie et al., 1994; Smith et al., 1995; Lisle & Rose, 1995; Karanis & Seitz, 1996; Karanis et al., 1996a; Widmer et al., 1996). Animal reservoirs inhabiting areas in surrounding of the surface water supplies are a potential risk factor. Oocysts of *Cryptosporidium* spp. can survive outside the hosts for about two years. Transmission occurs directly by faecal-oral transfer between humans or indirectly through the ingestion of contaminated food or water, including cold or pristine water. Surface water reservoirs can become contaminated with *Cryptosporidium* oocysts by domestic and wild animals and human sewage. Reports have suggested that several animals could be sources of water-borne *Cryptosporidium* contamination, in particular cattle (Lisle & Rose, 1995; Smith et al., 1995); more than 40 different animal species have been reported to harbour *Cryptosporidium* infections (Current, 1988; Fayer & Ungar, 1986). Six species of *Cryptosporidium* have been recognized: *C. parvum*, *C. muris*, *C. baileyi*, *C. meleagrisidis*, *C. nasorum* and *C. crotali* (Current, 1988; Smith et al., 1995).

In this report we describe the prevalence of *Cryptosporidium* oocysts in faecal samples of muskrats trapped in different locations in Germany close to drinking water reservoirs.

**MATERIALS AND METHODS**

Studies on natural infections of muskrats (*Ondatra zibethica*) with *Cryptosporidium* were carried out in two geographically different locations in Germany. The animals were caught using special traps as described by Karanis et al., 1996b for *Giardia* studies, and one sample of each animal was collected. The faeces of each muskrat were tested once.
Purification procedure of Cryptosporidium oocysts

Oocysts were separated from faecal samples using sucrose – density – gradient centrifugation as described in previous reports (Roberts-Thompson, 1976; Renoth et al., 1996; Karanis et al., 1996c). The faeces were emulsified, seeded and allowed to settle overnight. Aliquots of the sediment were layered on 1 M sucrose solution and centrifuged at 500 x g for 15 min. The oocysts collected in the interface between the sucrose solution and the aliquot were removed and washed twice in distilled water (centrifugation in 1,000 x g for 5 min). The presence of Cryptosporidium oocysts was verified by direct immunofluorescence test (Crypto-Giardia-CELL®, Cellabs Pty Ltd Sydney Australia) which was performed according to the manufacturers instructions, and has been described in previous publications (Renoth et al., 1996; Karanis & Seitz 1996; Karanis et al., 1996b). All the slides were examined at x 400.

Samples were considered positive if one or more oocysts were observed. Identification was based on the size and morphology of the oocysts. Cryptosporidium parvum oocysts are round in shape, measuring 5 μm in diameter. After staining with a specific monoclonal antibody, they show bright apple-green fluorescence.

Results

Table I shows the results of the examinations by direct immunofluorescence (DIF) for Cryptosporidium oocysts in muskrats from the investigation areas A and B. Out of a total 121 trapped animals, faecal samples from 56 (46.3 %) were positive for Cryptosporidium oocysts after DIF. All muskrats (100 %, n = 16) from area A were positive, and 40 out of 105 animals (38.1 %) from area B were positive. The number of the oocysts detected by this procedure has not been determined. Within this qualitative study all detected Cryptosporidium parasites were identified as C. parvum species according to morphological characteristics of the oocysts.

<table>
<thead>
<tr>
<th>Number of animals examined</th>
<th>Animals infected with Cryptosporidium</th>
<th>Cryptosporidium positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>105</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>56</td>
</tr>
</tbody>
</table>

Table I. — Cryptosporidium infections in muskrats from area A and B. Identification was carried out by DIF.

Discussion

Infections with Cryptosporidium in muskrats were observed in 46.3 % of the examined animals. Several other studies have indicated a high prevalence of Cryptosporidium in cattle and other domestic animals and suggested that these are reservoirs of the disease (Benett et al., 1985; Upton & Current, 1985; Xiao & Herd, 1994). The host specificity of Cryptosporidium spp. is broad: it infects a wide range of animals, as well as humans (Nime et al., 1976; Hering et al., 1989; Moore & Zeman, 1991; Lindsay et al., 1991; Ditrich et al., 1991; Scott et al., 1994; Krause et al., 1995; Scott et al., 1995). However it is questionable whether the Cryptosporidium strains commonly found in animals are the same as those infecting humans. There has been some evidence of sequence heterogeneity among Cryptosporidium isolates derived from human and animal sources (Carraway et al., 1996; Widmer et al., 1996). More research on the infectivity and virulence of Cryptosporidium strains in humans is therefore required.

Animals have been implicated in outbreaks of waterborne cryptosporidiosis in humans (Smith & Rose, 1990; Mackenzie et al., 1994). Cryptosporidium has been found in 44.7 % of surface (raw) water samples from six different water treatment plants in Germany (Karanis & Seitz, 1996), in 75.6 % of surface water supplies in the USA and in 21.4 % of surface water supplies in the United Kingdom (Lisle & Rose, 1995). Because of the significance of the water-borne Cryptosporidium infections it would be desirable for water treatment authorities to be able to distinguish both possible sources of contamination, and whether the contaminating oocysts represent species which could give rise to infections in humans.

In the present study it has been demonstrated that a significant proportion in the vicinity of the drinking water supply areas in Germany harbour C. parvum infections, and that these animals could represent a source of water contamination.

It remains to be investigated whether these C. parvum isolates could be infective to humans. Comparison of the genetic features of Cryptosporidium isolates from water supplies, sewage discharges and the faeces of domestic and wild animals will help to determine the sources of contamination and will permit the water industries, which treat surface water for drinking purposes to evaluate the risk to the supply, and take measures to protect water supplies from the contamination with Cryptosporidium oocysts.

References


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