CRYPTOSPORIDIUM INFECTIONS IN MUSKRAT (*ONDATRA ZIBETHICA*)

PETRI C.*, KARANIS P.* & RENOTH S.*

Summary :

The faeces of muskrats were examined for *Cryptosporidium* infections. A total of 121 muskrats trapped in areas close to surface water supplies in two different regions of Germany were examined for the presence of *Cryptosporidium* spp. oocysts. Faecal samples from these animals were homogenised and processed by sucrose-density-gradient centrifugation, through a 1 M sucrose solution. Separated oocysts were identified by immunofluorescence test assay. 56 animals (46.3 %) were found to be *Cryptosporidium* positive. These results implicate muskrats as a source of contamination of surface water supplies with *Cryptosporidium* oocysts.

KEY WORDS : *Cryptosporidium*, diagnosis, immunofluorescence, muskrats, water supplies, contamination.

Résumé : INFECTIONS À *CRYPTOSPORIDIUM* CHEZ LE RAT MUSQUÉ (*ONDATRA ZIBETHICA*)

Les matières fécales de rats musqués ont été examinées pour la recherche d'oocystes de Cryptosporidium *spp. Au total, 121 animaux ont été piégés près de points d'eau de surface, dans deux régions différentes d'Allemagne. Les échantillons de fèces ont été homogénéisés et centrifugés en gradient de densité en utilisant une solution de sucrose 1 M. Les oocystes séparés ont été indentifiés par immunofluorescence. 56 animaux (46,3 %) se sont révélés excréteurs d'oocystes de* Cryptosporidium. *Ces résultats montrent que les rats musqués peuvent être une source de contamination des eaux de surface avec des oocystes de cryptosporidies.*

MOTS CLÉS : Cryptosporidium, *diagnostic, immunofluorescence, rat musqué, eaux de surface, contamination.*

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.*, 1996*a*; 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. meleagridis*, *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.*, 1996*b* for *Giardia* studies, and one sample of each animal was collected. The feaces of each muskrat were tested once.

* Institut für Medizinische Parasitologie, Universität Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany.
Correspondence : Panagiotis Karanis. Tel : 0049-(0)228-287-4336. Fax : 0049-(0)228-287-4330. e-mail:karanis@parasit.meb.uni-bonn.de

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.*, 1996*c*). 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 × 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 × 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 manufactures instructions, and has been described in previous publications (Renoth *et al.*, 1996; Karanis & Seitz 1996; Karanis *et al.*, 1996*b*). All the slides were examined at × 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.

	Number of animals examined	Animals infected which *Cryptosporidium*	*Cryptosporidium* positive (%)
A	16	16	100
B	105	40	38.1
Total	121	56	46.3

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 water-borne 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

BENNETT M., BAXBY D., BLUNDELL N., GASKELL C.J. & KELLY D.F. Cryptosporidiosis in the domestic cat. *Veterinary Record*, 1985, *116*, 73.

CARRAWAY M., TZIPORI S. & WIDMER G. Identification of genetic heterogeneity in the *Cryptosporidium parvum* ribosomal repeat. *Applied and Environmental Microbiology,* 1996, *62,* 712-716.

CURRENT W.L. The biology of *Cryptosporidium. ASM News,* 1988, *54,* 605-611.

DITRICH O., PALKOVIC L., STERBA J., PROKOPIC J., LOUDOVA J. & GIBODA M. The first finding of *Cryptosporidium baileyi* in man. *Parasitology Research,* 1991, *77,* 44-47.

FAYER R. & UNGAR B.L. *Cryptosporidium* spp. and cryptosporidiosis. *Microbiological Reviews,* 1986, *50,* 458-483.

HANSEN J.S. & ONGERTH J.E. Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts in river water. *Applied and Environmental Microbiology,* 1991, *57,* 2790-2795.

HAYES E.D., MATTE D.T., O'BRIEN R.T., MCKINLEY W.T., LOGSDON S.G., ROSE J.B., UNGAR B.L.P., WORD D.M., PINSKY P.F., CUMMINGS M.L., WILSON M.A., LONG E.G., HURWITZ E.S. & JURANEK D. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. *New England Journal of Medicine,* 1989, *320,* 1372-1376.

HERING L., JUNGMANN R., ELIEWSKI U. & MARCHLEWITZ B. Querschnittsuntersuchungen zum Vorkommen von Kryptosporidien- Infektionen-eine 2-Jahres-Studie. *Zeitschrift für Klinische Medizin,* 1989, *18,* 1535-1539 (in German with English summary).

KARANIS P. & SEITZ H.M. Vorkommen und Verbreitung von *Giardia* und *Cryptosporidium* in Roh- und Trinkwasser von Oberflächen-wasserwerken. *gwf.Wasser.Abwasser,* 1996, *Heft 2,* 94-100 (in German with English summary).

KARANIS P., SCHOENEN D. & SEITZ H.M. *Giardia* and *Cryptosporidium* in backwash water from rapid sand filters using for drinking water. *Zentralblatt für Bakteriologie,* 1996*a*, *284,* 107-114.

KARANIS P., OPIELA K., AL-AROUSI M. & SEITZ H.M. A comparison of phase contrast microscopy and an immunofluorescence test for the detection of *Giardia* spp. in faecal specimens from cattle and wild rodents. *Transactions of Royal Society of Tropical Medicine and Hygiene,* 1996*b*, *90,* 250-251.

KARANIS P., OPIELA K., RENOTH S. & SEITZ H.M. Possible contamination of surface waters with *Giardia* spp. through muskrats. *Zentralblatt für Bakteriologie,* 1996*c*, *284,* 302-306.

KRAUSE W., ABRAHAM A. & LEHMAN D. Cryptosporidiosis bei Kindern mit Enteritis-Symptomatik aus dem Regierungsbezirk Leipzig 1987-1992. *Cryptosporidium* spp. in diarrhoic children from the Leipzig district 1987-1992. *Applied Parasitology,* 1995, *36,* 67-71 (in German with English summary).

LECHEVALLIER M.W., NORTON W.D., LEE R.G. & ROSE J.B. Detection and treatment of *Giardia* and *Cryptosporidium* in water supplies. *AWWA Research Foundation,* 1991, *6666 West Qincy Avenue Denver, CO 80235.*

LINDSAY D.S., BLAGBURN B.L., HOERR F.J. & SMITH P. Cryptosporidiosis in zoo and pet birds. *Journal of Protozoology,* 1991, *6,* 180S-181S.

LISLE J.T. & ROSE J.B. *Cryptosporidium* contamination of water in the USA and UK: a mini-rewiew. *Journal Water STR-Aqua,* 1995, *44,* 103-117.

MACKENZIE W.R., HOXIE N.J., PROCTOR M.E., GRADUS M.S., BLAIR K.A., PETERSON D.E., KAZMIERCZAK J.J., ADDISS D.G., FOX K.R., ROSE J.B. & DAVIS J.P. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine,* 1994, *331,* 161-167.

NIME F.A., BUREK J.D., PAGE D.L., HOLCHER M.A. & YARDLEY J.H. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium. Gastroenterology,* 1976, *70,* 592-598.

MOORE D.A. & ZEMAN D. Cryptosporidiosis in neonatal calves: 277 cases (1986–1987). *JAVMA,* 1991, *11,* 1969-1970.

ONGERTH J.E. & STIBBS H.H. Identification of *Cryptosporidium* oocysts in river water. *Applied and Environmental Microbiology,* 1987, *53,* 672-676.

RENOTH S., KARANIS P., SCHOENEN D. & SEITZ H.M. Recovery efficiency of *Cryptosporidium* from water with a crossflow system and continuous flow centrifugation: a comparison study. *Zentralbaltt für Bakteriologie,* 1996, *283,* 522-528.

RICHARDSON A.J., FRANKENBERG R.A., BRUCK A.C., SELKON J.B., COLBOURNE J.S., PARSON J.W. & MAYON-WHITE R.T. An outbreak of waterborne Cryptosporidiosis in Swindown and Oxfordshire. *Epidemiology and Infection,* 1991, *107,* 485-495.

ROBERTS-THOMPSON I.C., STEVENS D.P., MAHMOUD A.A.F. & WARREN K.S. Giardiasis in the mouse: an animal model. *Gastroenterology,* 1976, *71,* 57-61.

ROSE J.B., KAYED D., MADORE M.S., GERBA C.P., ARROWOOD M.J., STERLING G.C.R. & RIGGS J.L. Methods for the recovery of *Giardia* and *Cryptosporidium* from environmental waters and their comparative occurrence, *in :* P.M. & Hammond B.R. (eds), Advances in *Giardia* Research. Wallis, , The University of Calgary Press,Calgary, 1988, 205-209.

SCOTT C.A., SMITH H.V. & GIBBS H.A. Excretion of *Cryptosporidium parvum* in herd of beef suckler cows. *Veterinary Record,* 1994, *12,* 172.

SCOTT C.A., SMITH H.V., MTAMBO M.M.A. & GIBBS H.A. An epidemiological study of *Cryptosporidium parvum* in two herds of adult beef cattle. *Veterinary Parasitology,* 1995, *57,* 277-288.

SMITH H.V. & ROSE J.B. Waterborne Cryptosporidiosis. *Parasitology Today,* 1990, *6,* 8-12.

SMITH H.V., ROBERTSON L.J. & ONGERTH J.E. Cryptosporidiosis and Giardiasis: the impact of waterborne transmission. *Journal Water SRT-Aqua,* 1995, *44,* 258-274.

UPTON J.S. & CURRENT W.E. The species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) infecting mammals. *Journal of Parasitology,* 1985, *71,* 625–629.

WIDMER G., CARRAWAY M. & TZIPORI S. Waterborne Cryptosporidiosis: A perspective from the USA. *Parasitology Today,* 1996, *7,* 286-290.

XIAO L. & HERD R.P. Review of equine *Cryptosporidium* infection. *Equine Veterinary Journal,* 1994, *26,* 9-13.

Reçu le 5 février 1997
Accepté le 3 juillet 1997