Acta Tropica 80 (2001) 145–149

ACTA TROPICA

www.parasitology-online.com

Occurrence of *Cryptosporidium* sp. oocysts in fecal and water samples in Austria

Andreas Hassl [a,c,*], Gerald Benyr [b], Regina Sommer [c]

[a] *Micro-Biology Consult Andreas Hassl, Ameisgasse 63/4/12, A-1140 Vienna, Austria*
[b] *Natural History Museum Vienna, Burgring 7, A-1014 Vienna, Austria*
[c] *Clinical Institute of Hygiene, University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria*

Received 29 May 2000; received in revised form 13 November 2000; accepted 15 January 2001

Abstract

Oocysts of *Cryptosporidium* spp. were detected and differentiated by a modular arranged gene amplification procedure in various samples, mostly human stool, feces of herpetotaxa, and water, in different locations of South and Eastern Austria. *Cryptosporidium parvum* was found in stool samples of immunocompromised persons, in reptile feces, and in water samples. The presence of *Cryptosporidium* in an area is probably associated with high human population densities since water from protected sources in sparsely inhabited areas is rarely contaminated. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Cryptosporidium* sp.; Parasite; Pet herpetotaxa; Water; Austria

1. Introduction

Cryptosporidia (*Cryptosporidium* spp.) are obligatory parasitic protozoa of different vertebrates including man; infections cause severe diarrhea and may lead to death in immunocompromised hosts. Although the waterborne nature of several human cases is well known and documented (Goldstein et al., 1996), infection routes and the reservoirs in the wilderness, which may consist of lower vertebrates (Graczyk et al., 1998) are still unknown to a great extent. Reasons include lack of simple and reliable methods for species identification and considerable uncertainty about the virulence of different *Cryptosporidium* species in different hosts (Graczyk et al., 1996). Reptiles have been found to be infected with *Cryptosporidium serpentis* (Graczyk and Cranfield, 1996), and they may also disseminate oocysts of other species (Arcay et al., 1995). Pet animals may act as vectors or as reservoirs, and they may acquire their infections from man or from other mammals.

Since 1984, cryptosporidiosis caused by *Cryptosporidium parvum* is regularly diagnosed in Austrian AIDS patients (Aspoeck and Hassl, 1990). But no facts are known about the distribution of this parasite, about the incidence of cryptosporid-

* Corresponding author. Present address: Kinderspitalgasse 15, A-1095 Vienna, Austria. Tel.: +43-664-2302413; fax: +43-1-403834390.

E-mail address: andreas.hassl@univie.ac.at (A. Hassl).

PII: S0001-706X(01)00169-3

iosis in cattle and companion animals, and about the frequency of *Cryptosporidium* contamination in water samples. Accordingly, human stool samples, fecal samples from reptiles, amphibians, and mice, and samples from water sources and water for human consumption were tested for the presence of oocysts of *Cryptosporidium* spp.

2. Materials and methods

Samples were collected at different locations in the eastern parts of Austria in the period from January 1998 till the first week of 2000. Four hundred and thirty-seven human stool samples, 429 of them from HIV1-infected persons, were examined during a medical surveillance. Four hundred and fifty feces samples were collected from captive reptiles and amphibians of various taxa usually kept as pets (frogs 20%; snakes 25%; lizards 47%; turtles 8%). Twenty-nine feces samples of free-living lizards (mostly *Lacerta horvathi*) were collected during excursions to nature reserve areas. In addition, 24 feces samples from feeding mice bred in professional institutions were tested. Water samples of about 150 l each (109 from drinking water reservoirs before treatment, 11 samples of water for human consumption) were processed according to Graczyk et al. (1997b). A modular arranged procedure for the detection of oocysts of different *Cryptosporidium* species (*C. parvum* 'human', 'calf'; *C. serpentis*; *C. muris*) in various samples has been developed previously (Hassl et al., 1999), and was applied to this study. The core component was a gene amplification technique according to Morgan et al. (1997) in combination with a microscopical examination, species assignment was done by amplificon length determination.

3. Results

Oocysts of different *Cryptosporidium* species were detected in water samples, in human stool, and in feces of exotic pets. The frequency of oocyst appearance in the samples is shown in Table 1, the seasonal variance of the appearance is presented in Fig. 1. No enlarged oocysts were found in feeding mice and in free-living reptiles. Moreover, no relation was found between oocyst shedding in the herpetotaxa and the type of feeding or the type of terraria. The locations of oocyst detection in correlation with the human population density is presented in Fig. 2.

4. Discussion

Environmental *Cryptosporidium* contamination, especially the contamination of drinking water, has not been determined in Austria, and the transmission route and the frequency of a transmission between man and animals are under debate. Our data indicate a relatively high contamination rate of water with *Cryptosporidium* oocysts other than

Table 1
Detection of *Cryptosporidium* oocysts in various samples in Austria

Sample	*n*	Oocysts detected (*n*)	Positive (%)	*Cryptosporidium* species (%)
Human stool	437	19	4.3	*C. parvum* (100)
Feces of captive herpetotaxa	450	21	4.6	*C. parvum* (14); *C. serpentis* (24); species undetermined (62)
Feces of free-living lizards	29	0	0	
Feces of mice	24	0	0	
Water from sources	109	11	10	*C. parvum* (18); species undetermined (82)
Water for human consumption	11	0	0	

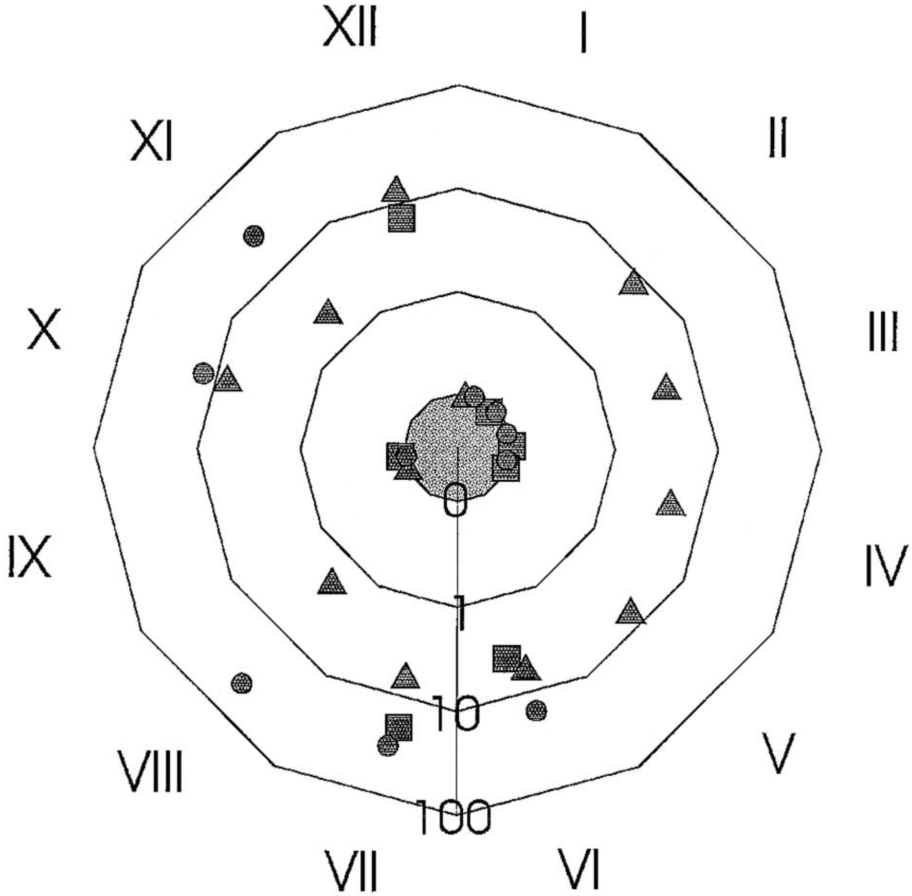

Fig. 1. Seasonality of *Cryptosporidium* oocyst presence in Austria. Roman numbers: month; numbers and dodecagons: percent of positive samples on a logarithmic scale; geometric figure symbolize the type of samples: circle: water; square: human stool; triangle: feces.

C. parvum. These species are technically difficult to determine (Xiao et al., 1999; Morgan et al., 1995, 1999) primarily due to lack of distinctive features; these species, however, seem to be of minor importance to human health (Xiao et al., 2000).

C. parvum is regularly detected in stool samples from HIV-infected persons. There has been a slight decrease in the number of human infections since 1990: 9.2% of the Austrian HIV1-infected persons shed oocysts at that time (Aspoeck and Hassl, 1990), now only 4.3% shed oocysts. This observation, however, may be due to a shift in the composition of the patients cohort caused by fewer AIDS patients at present time, as *Cryptosporidium* is the cause of 15.6% cases of the diarrhea in patients with fully developed AIDS (Lopezvelez et al., 1995).

While amphibians are rarely associated with *Cryptosporidium* infections, pet frogs are known to excrete oocysts of an undetermined species (Hassl, 1991). Exotic pet animals, amphibians and reptiles, can shed oocysts of more than one *Cryptosporidium* species, sometimes even infectious *C.*

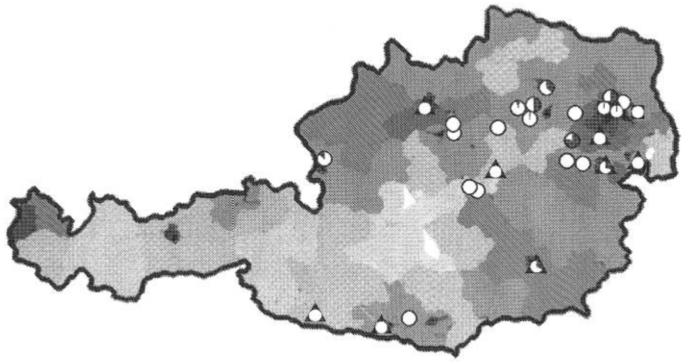

Fig. 2. Locations of *Cryptosporidium* oocysts in Austria. Shaded parts: human population density: light: < 25 persons/km^2; dark: > 200 persons/km^2. Geometric figure symbolize the type of samples: circle: water; square: human stool; triangle: feces. Inner pie chart: part of positive samples.

parvum oocysts (Arcay et al., 1995). Although this has been queried (Graczyk et al., 1996), the same workers suggest that oocysts from lower vertebrates can contaminate water sources (Graczyk et al., 1997a). Our data indicate a low infection rate of free-living lower vertebrates, i.e. lizards living in nature reserve areas. The source of the infection in captive herpetotaxa is unclear, but an anthropogenic origin seems likely. Immunodeficiency due to advanced age of specimens kept in terraria may be a factor that favors infection. Nevertheless, immunodeficient persons should be aware of this hazard in keeping exotic pets in terraria; to at least 90 000 Austrian households are believed to keep such pets.

As reported by Sorvillo et al. (1998), we found a two-culminated seasonal peak in the occurrence of *Cryptosporidium* oocysts. The reason for this remains unclear, particularly since infection incidence rates in animals do not follow this pattern. In Austria, the frequency of oocyst presence is related to the human population density rather than to the intensity of cattle breeding.

Acknowledgements

The authors thank Dr Werner Mayer (NHM Vienna) for organizational support, the Austrian Herpetological Society for scientific support, Mrs Ingrid Blöschl and Mrs Ilse Veits for excellent technical assistance.

References

Arcay, L., DeBorges, E.B., Bruzual, E., 1995. Criptosporidiosis experimental en la escala de vertebrados. I. Infecciones experimentales, II. Estudio histopathologico. Parasitol. al Dia. 19, 20–29.

Aspoeck, H., Hassl, A., 1990. Parasitic infections in HIV patients in Austria: first results of a long-term study. Zbl. Bakt. Hyg. 272, 540–546.

Goldstein, S.T., Juranek, D.D., Ravenholt, O., Hightower, A.W., Martin, D.G., Mesnik, J.L., Griffiths, S.D., Bryant, A.J., Reich, R.R., Herwaldt, B.L., 1996. Cryptosporidiosis: an outbreak associated with drinking water despite state-of-the-art water treatment. Ann. Internal Med. 124, 459.

Graczyk, T.K., Cranfield, M.R., 1996. Assessment of the conventional detection of fecal *Cryptosporidium serpentis* oocysts in subclinically infected captive snakes. Vet. Res. 27, 185–192.

Graczyk, T.K., Fayer, R., Cranfield, M.R., 1996. *Cryptosporidium parvum* is not transmissible to fish, amphibians, or reptiles. J. Parasitol. 82, 748–751.

Graczyk, T.K., Fayer, R., Cranfield, M.R., 1997a. Zoonotic transmission of *Cryptosporidium parvum*. Implications for water-borne cryptosporidiosis. Parasitol. Today 13, 348–351.

Graczyk, T.K., Cranfield, M.R., Fayer, R., 1997b. Recovery of waterborne oocysts of *Cryptosporidium* from water samples by the membrane-filter dissolution method. Parasitol. Res. 83, 121–125.

Graczyk, T.K., Cranfield, M.R., Mann, J., Strandberg, J.D., 1998. Intestinal *Cryptosporidium* sp. infection in the Egyptian tortoise *Testudo kleinmanni*. Int. J. Parasitol. 28, 1885–1888.

Hassl, A., 1991. An asymptomatic Cryptosporidia (Apicomplexa: Coccidia) infection in *Agalychnis callidryas* (COPE, 1862) (Anura: Hylidae). Herpetozoa 4, 127–131.

Hassl, A., Vorbeck-Meister, I., Sommer, R., Rotter, M., 1999. Detection and identification of Cryptosporidia in faeces, stool and environmental samples. Mitt. Österr. Ges. Tropenmed. Parasit. 21, 45–50.

Lopezvelez, R., Tarazona, R., Camacho, A.G., Gomezmampaso, E.G., Guerrero, A., Moreira, V., Villanueva, R., 1995. Intestinal and extraintestinal cryptosporidiosis in AIDS patients. Eur. J. Clin. Microbiol. Infect. Dis. 14, 677–681.

Morgan, U.M., Constantine, C.C., Odonoghue, P., Meloni, B.P., Obrien, P.A., Thompson, R.C.A., 1995. Molecular characterization of *Cryptosporidium* isolates from humans and other animals using random amplified polymorphic DNA analysis. Am. J. Trop. Med. Hyg. 52, 559–564.

Morgan, U.M., Constantine, C.C., Forbes, D.A., Thompson, R.C., 1997. Differentiation between human and animal isolates of *Cryptosporidium parvum* using rDNA sequencing and direct PCR analysis. J. Parasitol. 83, 825–830.

Morgan, U.M., Monis, P.T., Fayer, R., Deplazes, P., Thompson, R.C., 1999. Phylogenetic relationships among isolates of *Cryptosporidium*: evidence for several new species. J. Parasitol. 85, 1126–1133.

Sorvillo, F., Beall, G., Turner, P.A., Beer, V.L., Kovacs, A.A., Kraus, P., Masters, D., Kerndt, P.R., 1998. Seasonality and factors associated with cryptosporidiosis among individuals with HIV infection. Epidemiol. Infect. 121, 197–204.

Xiao, L., Sulaiman, I., Fayer, R., Lal, A.A., 1999. Species and strain specific typing of *Cryptosporidium* parasites in clinical and environmental samples. Mem. Inst. Oswaldo. Cruz 93, 687–691.

Xiao, L., Morgan, U.M., Fayer, R., Thompson, R.C.A., Lal, A.A., 2000. *Cryptosporidium* systematics and implications for public health. Parasitol. Today 16, 287–292.