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Infectivity of *Cryptosporidium parvum* oocysts is retained upon intestinal passage through a migratory water-fowl species (Canada goose, *Branta canadensis*)

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Summary Five *Cryptosporidium*-free Canada geese (*Branta canadensis*) were individually orally dosed with 3.5×10^6 *Cryptosporidium parvum* oocysts infectious to neonatal BALB/c mice. After intestinal passage, inoculum-derived oocysts extracted from goose faeces established severe infection in 14 neonatal BALB/c mice (inoculum dose 2.5×10^5/mouse). The inoculum-derived oocysts were detected in goose faeces up to 9 days post-inoculation (PI); the number of intact oocysts and oocyst shells shed during the first 3 days PI was significantly higher than for the remaining 6 days PI ($P<0.01$). Based on acid-fast stained air-dried direct wet smears, 62% of the oocysts in goose faeces were intact (oocyst shells constituted 38%) and conformed to morphological features of viable and infectious inoculum oocysts. The fluorescence scores of the inoculated oocysts, obtained by use of the MERIFLUOR test, were identical to those obtained for the faeces-recovered oocysts (majority 3+ to 4+). The dynamics of oocyst shedding showed that overall, the birds released a significantly higher number of intact oocysts than oocyst shells ($P<0.01$). Retention of the viability and infectivity of *C. parvum* oocysts following intestinal passage through a migratory water-fowl species has serious epidemiological implications. Water-fowl can serve as mechanical vectors for the water-borne oocysts and can contaminate surface waters with *C. parvum*. As the concentration of *Cryptosporidium* oocysts in source waters is attributable to water-shed management practices, water-shed protection programme officials should consider water-fowl as a potential factor enhancing contamination of the source water with *Cryptosporidium*.

keywords *Cryptosporidium parvum*, water-borne oocysts, water contamination, Canada goose, *Branta canadensis*, water-fowl

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Introduction

Cryptosporidium parvum infects 79 mammalian species (Fayer *et al.* 1997) and is readily transmissible to humans. The infection significantly contributes to the mortality of immunocompromised or immunosuppressed individuals (Goodgame *et al.* 1993) and may severely debilitate immunocompetent people (O'Donoghue 1995). Transmissible via water, the pathogen represents a global public health threat

(Anonymous 1994), responsible for numerous recreational and drinking water outbreaks (Lisle & Rose 1995). *Cryptosporidium* infections constitute a major cause of gastrointestinal illness in non-outbreak settings (Moore *et al.* 1994).

Waterborne transmission is enhanced by the oocyst's small size (3.5–5.5 μm), long-lasting infectivity in the environment, resistance to standard water disinfection procedures applied to control other water-borne pathogens, and suboptimal treatment of source water by water treatment facilities (Lisle & Rose 1995). *Cryptosporidium* oocysts are frequently present in surface waters (Hansen & Ongerth 1991) and adverse weather conditions (heavy rains, snow melts and floods) contribute to water contamination by washing oocysts from the land into surface waters, elevating turbidity, causing sewage overflow and increasing urban and agricultural run-off (Lisle & Rose 1995). Water-shed protection programmes include elimination of human activity from the area of water catchment (Lisle & Rose 1995). However, waters from protected watersheds have been as contaminated as those which are unprotected or affected by industry (Rose *et al.* 1997), raising the concern that environmental factors contributing to contamination are not fully recognized (Lisle & Rose 1995).

Cryptosporidium infections have been reported in free-ranging animals (Martin & Zeider 1992; Elanbaum *et al.* 1993; Fayer *et al.* 1996), and wildlife was postulated as a potential factor contributing to water contamination (Hansen & Ongerth 1991). *Cryptosporidium parvum* is non-infectious to lower aquatic and semi-aquatic vertebrates, but those animals can act as mechanical vectors and disseminate ingested oocysts (Graczyk *et al.* 1996a). Pastures and grazing lands are recognized as significant sources of viable C. *parvum* oocysts (Coleman *et al.* 1989; Hiepe & Buchwalder 1991; Lisle & Rose 1995) and during spring and fall migrations, thousands of water-fowl use these areas for feeding and resting (Fisher 1979). Passage of infectious C. *parvum* oocysts through the gastrointestinal tract of a refractory avian host (Peking duck, *Anas platyrhynchos*) does not alter their infectivity for mammalian hosts (Graczyk *et al.* 1996b). Aquatic birds, which have unlimited access to surface waters, can therefore act as mechanical vectors by disseminating ingested oocysts. Although a free-ranging strain of Peking ducks (Mallards, *A. platyrhynchos*) occurs in the wild, it needs to be substantiated how the findings characteristic for Peking ducks are applicable for typical and the most prevalent species of water-fowl such as the Canada goose (*Branta canadensis*) (Fisher 1979; Anonymous 1996).

The purpose of the present study was to determine whether *C. parvum* oocysts will retain their infectivity after intestinal passage through Canada geese. This species was selected because of its abundance in the wild (Anonymous 1996). It is the most representative species of Anatidae, constituting a major component of water-fowl migration along the east coast of the United States (Fisher 1979; Anonymous 1996).

Materials and methods

Cryptosporidium parvum (AUCP-1 strain) oocysts were extracted from the faeces of experimentally infected Holstein calves (Fayer & Ellis 1993), purified by caesium chloride (CsCl) gradient centrifugation (Kilani & Sekla 1987) and stored for 2 weeks at 4°C in potassium dichromate ($K_2Cr_2O_7$). The oocysts were washed 5 times with phosphate buffered saline (PBS) (pH 7.4) by centrifugation (750 g, 5 min) at 4°C to remove $K_2Cr_2O_7$. Oocysts were counted with the aid of a haemacytometer (Fayer & Ellis 1993), morphologically assessed by modified acid-fast stain (AFS) of the air-dried direct wet smear (DWS) (Ash & Orihel 1987), and infectivity was tested by gastric intubation of 10 neonatal BALB/c mice (Fayer *et al.* 1995).

Five 2–3-year-old Canada geese (*Branta canadensis*) were obtained from the Wildlife Rehabilitation and Education Center (Elkridge, Maryland, USA). The birds were transferred to the Baltimore Zoological Society (Baltimore, Maryland, USA) and housed indoors in a room (15 m × 10 m) with a tap water swimming pool and pelleted food (Purina, Purina Mills Inc., St Louis, Missouri, USA) *ad libitum*. Using the Argyle Premature Infant Feeding Set (Sherwood Medical, A Brunswick Company, St Louis, Missouri, USA), each goose was gastrically intubated with 1.5 ml of PBS containing 3.5×10^6 C. *parvum* oocysts. Geese were released to the Wildlife Rehabilitation and Education Center following experimentation.

Faeces was collected daily 3 days prior to inoculation and for 16 days post-inoculation (PI). As the geese were housed together, faecal specimens contained stools of all 5 animals. Prior to inoculation, faeces was examined for *Cryptosporidium* oocysts by AFS DWS, Sheather's sugar coverslip flotation (SSCF) (Ash & Orihel 1987), and the MER*IFLUOR* *Cryptosporidium/Giardia* (Meridian Diagnostic, Inc., Cincinnati, Ohio, USA) test kit for direct immunofluorescence (Graczyk *et al.* 1995). Post-inoculation faecal specimens were weighed and mixed homogeneously with 10% (w/v) of PBS (pH 7.4). Ten AFS DWS were prepared. Each was examined for 10 min by light microscopy, and the number of oocyst shells and intact oocysts counted. The percentage of oocyst shells to all recovered oocysts was computed on the basis of the mean number obtained from 10 counts and adjusted for the 1/10 dilution. Of each faecal specimen collected 1, 2 and 3 days PI, 100 g was subjected to SSCF. The SSCF-recovered material was examined by AFS in the manner presented above and by immunofluorescence microscopy using the MER*IFLUOR* test. Examination of slides and scoring of fluorescence followed the previous protocol (Garcia *et al.* 1992). Faecal specimens positive for oocysts by AFS DWS and MER*IFLUOR* test were used for oocyst extraction (Graczyk & Cranfield 1996) and purification conducted at 4°C by CsCl gradient centrifugation. The number of oocysts was assessed by both haemacytometer (Fayer & Ellis 1993) and the MER*IFLUOR* test (Garcia *et al.* 1992).

A total of 3.6×10^6 *C. parvum* oocysts extracted from geese faeces up to 3 days PI were used for the preparation of 14 vials with 2.5×10^5 oocysts per vial in 50 µl of PBS. Infectivity of faeces-recovered oocysts was determined by infectivity bioassay (Fayer 1995). Determination of oocyst viability using the 4′,6′-diamidino-2-phenylindole (DAPI) and propidium iodide (PI), or *in vitro* excystation, was not performed because DAPI/PI and *in vitro* excystation significantly overestimated the viability of oocysts compared to the infectivity bioassay (Finch *et al.* 1993; Black *et al.* 1996). Fourteen 5-day-old suckling BALB/c mice were individually inoculated by gastric intubation with 2.5×10^5 oocysts. Five 5-day-old suckling BALB/c mice inoculated with 50 µl of PBS served as control. The mice were euthanized (Fayer *et al.* 1995) on day 4 PI, and sections of ileum, caecum and colon were processed and histologically examined for development of *Cryptosporidium* intracellular stages (Fayer *et al.* 1995). The intensity of infection was scored as previously described (Fayer 1995).

Statistical analysis was performed with Statistix 4.1 (Analytical Software, St Paul, Minnesota, USA). The variables were examined by the Runs test to determine conformity to a normal distribution (Sokal & Rohlf 1981). The non-parametric Rank sum test was used to assess the significance of differences between variables. The *G*-heterogeneity test was used to assess differences between fractions. Mean values (x) were associated with standard deviation (s.d.). Statistical significance was considered to be $P \leqslant 0.05$.

Results

Faecal specimens collected from geese before inoculation with *C. parvum* oocysts were negative for *Cryptosporidium* oocysts by AFS DWS, SSCF, and the MER*IFLUOR* test.

Faecal specimens collected daily ranged in weight from 1100 to 1400 g ($\bar{x} = 1219 \pm 112$ g).

Acid-fast stained *C. parvum* oocysts from original inoculum, when examined by light microscopy, displayed a non-uniform, bright red coloration and a densely packed cytoplasm containing characteristic black granules. In contrast, oocyst shells (constituting approximately 15% of the oocysts overall) were pale, uniformly pink to light red-stained and did not contain black granules. The oocysts shed by the geese revealed these two variations in acid-fast-staining. Intact oocysts and oocyst shells conformed to the morphological features observed for the original inoculum oocysts. The oocyst shells constituted 38% of the oocysts released by the geese; 62% were intact.

The inoculum-derived oocysts were detected in goose faeces up to day 9 PI. The dynamics of shedding of intact oocysts and oocyst shells detected by the AFS DWS are presented in Figure 1. Counting of intact oocysts and oocyst shells in AFS DWS showed that (i), the number of intact oocysts and oocyst shells shed during the first 3 days PI was significantly higher than during the remaining 6 days PI (Rank

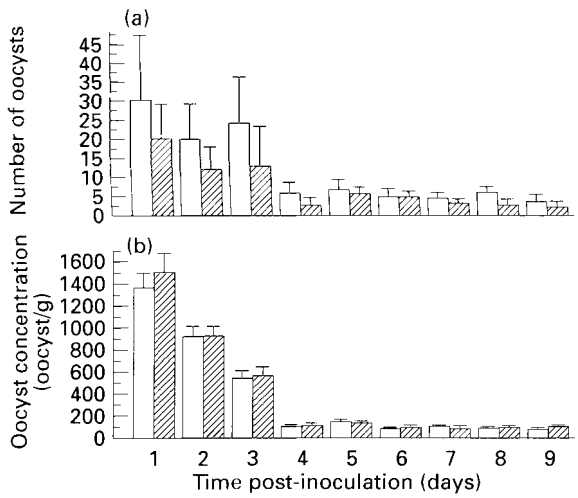

Figure 1 Temporal distribution of the mean number (± s.d.) of *Cryptosporidium parvum* oocysts shed in the faeces of 5 Canada geese (*Branta canadensis*) gastrically intubated with 3.5×10^6 of *C. parvum* (AUCP-1 strain) oocysts. a, Number of oocysts (□, intact oocysts; ▨, oocyst shells) determined by 10-minute examination of 10 acid-fast-stained direct wet smears; and b, faecal oocyst concentration determined by extraction of oocysts from faeces, purification by caesium chloride gradient centrifugation, and counted with □, haemacytometer and by ▨, immunodetection using the MER*IFLUOR* *Cryptosporidium/Giardia* test kit for direct immunofluorescence.

sum test $t=3.12$, $P<0.01$); (ii) overall, the geese shed a significantly higher number of intact oocysts than oocyst shells (Rank sum test $t=3.44$, $P<0.01$); and (iii), up to day 4 PI the number of intact oocysts was significantly higher than the number of oocyst shells (Rank sum test $t=1.97$, $P<0.05$).

Overall, oocyst shells constituted 38% of all goose-released oocysts, and 37% for the first 3 days PI when the oocyst output was most intense. The ratios of oocyst shells to all recovered oocysts were 40, 38, 35, 31, 45, 50, 42, 31 and 39% for the 9 consecutive PI days, respectively. Sheather's sugar cover-slip flotation and AFS examination of faecal specimens of days 1, 2 and 3 PI revealed both intact oocysts and oocyst shells at the ratios of 40, 34 and 21%, respectively. The ratios did not differ significantly compared to those of 46, 32 and 23% from faecal DWS of days 1, 2 and 3 PI, respectively (*G*-heterogeneity test; $G=0.21$, $P>0.05$). When the SSCF-recovered material was examined by the MER*IFLUOR* test, no difference was observed in the immunofluorescence of oocyst shells and intact oocysts. The fluorescence scores for *C. parvum* oocysts from the original inoculum were identical (majority 3+ to 4+) to those of oocysts recovered from goose faeces by SSCF.

The total number of oocysts recovered from goose faeces was 4.21×10^6 as determined by haemacytometer count and 4.37×10^6 by the MER*IFLUOR* test, representing 24 and 25%, respectively of the total of 1.8×10^7 oocysts administered as inoculum. The majority of oocysts (70% of total recovered) were released by geese during the first 3 days PI. Oocyst concentration significantly decreased on day 4 PI (Rank sum test; $t=2.42$, $P<0.01$) and all subsequent days through day 9 PI (Figure 1).

The ileum, caecum, and colon sections of all 14 neonatal BALB/c mice inoculated with *C. parvum* oocysts extracted from goose faeces contained large numbers of developmental stages of *Cryptosporidium*. The most severe infection was in the ileum, where >67% of the epithelial cells harboured developmental stages of the pathogen. The average score for the 14 mice was 65%. The control mice contained no developmental stages of *Cryptosporidium*.

Discussion

As Canada geese were negtive for *Cryptosporidium* oocysts prior to inoculation, and oocyst-positive for 9 consecutive days post-inoculation (PI), we conclude that the oocysts in faecal specimens originated from the inoculum. We further conclude that because *Cryptosporidium* oocysts were not detected by acid-fast-stain or MER*IFLUOR* test kit after day 9 PI, and since no oocysts were extracted from faeces collected after day 9 PI, it is unlikely that the pathogen had established infection in the geese. However, as demonstrated by the infectivity bioassay (Fayer 1995), the viability and infectivity of the inoculated *C. parvum* oocysts was retained after passage through the goose intestine.

Canada geese are abundant in the wild and constitute the majority of migratory water-fowl in the USA (Fisher 1979). As reported by the US Fish and Wildlife Service and Canadian Wildlife Service on Waterfowl Status for 1996, 12 populations of Canada geese migrate or reside within the United

States (Anonymous 1996). As of January 1996, the total number of birds, based on the estimate for each of 12 populations, exceeded 5 million (Anonymous 1996). In most of the Canada goose populations, the number of breeding pairs and non-breeding birds has increased for the 3 past years (Anonymous 1996). They use pastures and cattle grazing lands for feeding and resting during migration.

Considering the zoonotic transmission of *C. parvum* (Fayer *et al.* 1990), concentrations of domesticated animals, particularly dairy farms and cattle grazing lands, were identified as sources for viable oocysts which may contaminate surface waters (Lisle & Rose 1995). The importance of pastures in the epidemiology of *Cryptosporidium* was recognized after cider from fallen apples collected in a pasture caused an epidemic of cryptosporidiosis (Millard *et al.* 1994). The retention of infectivity of *C. parvum* oocysts following intestinal passage in the avian host representing the most abundant water-fowl species in the USA is epidemiologically important. As most of the water-fowl's daily activity involves grazing on land and shallow waters with defaecation into the water (Combs & Botzler 1991), Canada geese may act as mechanical vectors disseminating infectious oocysts in an aquatic environment. When a single dose of oocysts was administered to ducks (Graczyk *et al.* 1996b) and geese, the majority of oocysts were shed 48 and 72 h PI, respectively. Water-fowl which graze in pastures may ingest oocysts and subsequently shed the pathogen.

Heterologous cross-transmissions of *C. parvum* to avians in an attempt to establish infections were unsuccessful (O'Donoghue 1995), although in one experiment in 1 and 7-day-old chickens a weak, short-lasting tracheal (but not intestinal) infection could be established (Lindsay *et al.* 1987). In that experiment (Lindsay *et al.* 1987), the oocysts of tracheal origin were found in chicken faeces, providing evidence that *C. parvum* oocysts do not excyst in the avian digestive tract. The experiment on cross-transmission of *C. parvum* to Peking ducks (Graczyk *et al.* 1996b) and the present study confirmed this observation, as most of the viable and infectious *C. parvum* oocysts (73 and 68%, respectively) did not excyst during intestinal passage.

Although non-viable oocysts or viable but non-infectious oocysts are of minimal public health concern, the risk of water-borne cryptosporidiosis from infectious oocysts can be enormous (Lisle & Rose 1995). The United States Environmental Protection Agency recently issued a set of guidelines known as the Information Collection Rule (ICR) for monitoring influent water and finished drinking water for *Cryptosporidium* by any water treatment facility that serves more than 10 000 persons (Coley 1995). Our study offers useful information for facilitating the interpretation of the positive detection of oocysts at water treatment plants. First, if source water became contaminated with oocysts shed by water-fowl, because of their potential for movements and migrations, the timing of oocyst detection may not be conceived as related to or associated with water-fowl. Secondly, if oocysts shed by aquatic birds are detected at water treatment facilities by the immunofluorescence antibody (IFA) method (ASTM 1993), even non-infectious water-borne oocysts (i.e. oocyst shells) will produce a positive reaction. Thirdly, *C. parvum* is the only one of 8 valid species of *Cryptosporidium* (Fayer *et al.* 1997) which is infectious to humans. However, the oocysts of the remaining 7 medically unimportant species of *Cryptosporidium* have produced positive IFA reactions with commercially available test kits (Graczyk *et al.* 1996c). The oocysts recovered from water-fowl-contaminated waters will produce IFA reactions similar to *C. parvum* even if other species of *Cryptosporidium* are present.

A certain consistency was observed in the patterns of shedding by ducks of inoculum-derived oocysts (Graczyk *et al.* 1996b) and geese. The birds released the majority of oocysts within 2–3 days PI. In addition, the difference between the proportion of intact *C. parvum* oocysts to oocyst shells shed by ducks (73 and 27% respectively) (Graczyk *et al.* 1996b), and geese (62 and 38%, respectively) was not significant. Based on the results obtained for *Anas platyrhynchos* (Graczyk *et al.* 1996b) and *Branta canadensis*, we conclude that water-fowl, particularly migratory ducks and geese which may abundantly reside in water-shed areas, should be considered as a factor facilitating contamination of the water with *Cryptosporidium* oocysts. Oocyst concentration in source waters is attributable to water-shed management practices (Hansen & Ongerth 1991). Thus, modifications of water-shed

control activities regarding water-fowl may enhance prevention or reduce contamination, and decrease the number of cases associated with recreational contact with surface water.

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References

American Society for Testing and Materials (1993) *Proposed test method for* Giardia *cysts and* Cryptosporidium *oocysts in low turbidity water by a fluorescent antibody procedure.* In *Annual Book of ASTM Standards* 11.02. American Society for Testing and Materials, Philadelphia, pp. 925–935.

Anonymous (1994) Addressing infectious emerging disease threats: a prevention strategy for the United States. Executive summary. *Morbidity and Mortality Weekly Reports* **43**, 1–18.

Anonymous (1996) *Waterfowl population status.* U.S. Fish and Wildlife Service and Canadian Wildlife Service, pp. 35.

Ash LR & Orihel TC (1987) *Parasites: a guide to laboratory procedures and identification.* ASCP Press Inc., Chicago, IL, pp. 328.

Black EK, Fich GR, Taghi-Kilani R & Belosevic M (1996) Comparison of assays for *Cryptosporidium parvum* oocysts viability after chemical disinfection. *FEMS Microbiology Letters* **135**, 187–189.

Coleman SU, Klei TR, French DD, Chapman RR & Corstvet RE (1989) Prevalence of *Cryptosporidium* sp. in equids in Louisiana. *Australian Journal of Veterinary Research* **50**, 575–577.

Coley DG (1995) Waterborne cryptosporidiosis threat addressed. *Emerging Infectious Diseases* **1**, 67–68.

Combs MS & Botzler RG (1991) Correlations of daily activity with avian cholera mortality among wildfowl. *Journal of Wildlife Diseases* **27**, 543–550.

Elanbaum CS, Qualis C, Ewing SA & Lochmiller RL (1993) Cryptosporidiosis in a cotton rat (*Sigmodon hispidus*). *Journal of Wildlife Diseases* **29**, 161–164.

Fayer R (1995) Effect of sodium hypochlorite exposure on infectivity of *Cryptosporidium parvum* oocysts for neonatal BALB/c mice. *Applied and Environmental Microbiology* **61**, 844–846.

Fayer R, Dubey J P & Speer CA (1990) General biology of *Cryptosporidium*: In *Cryptosporidiosis in Man and Animals* (eds JP Dubey, CA Speer & R Fayer), CRC Press, Inc., Boca Raton, Florida, USA, pp. 1–29.

Fayer R & Ellis W (1993) Paromomycin is effective as prophylaxis for cryptosporidiosis in dairy calves. *Journal of Parasitology* **79**, 771–774.

Fayer R, Graczyk TK & Cranfield MR (1995) Multiple heterogenous isolates of *Cryptosporidium serpentis* from captive snakes are not transmissible to neonatal BALB/c mice (*Mus musculus*). *Journal of Parasitology* **80**, 482–484.

Fayer R, Speer CA & Dubey JP (1997) General biology of *Cryptosporidium* and cryptosporidiosis. In *Cryptosporidium and Cryptosporidiosis* (ed R Fayer), CRC Press, Inc., Boca Raton, Florida, USA, in press.

Fayer R, Fisher JR, Sewell CT, Kavanaugh DM & Osborn DA (1996) Spontaneous cryptosporidiosis in captive white-tailed deer (*Odocoileus virginianus*). *Journal of Wildlife Diseases* **32**, 619–622.

Finch GR, Black EK, Gyurek L & Belosevic M (1993) Ozone inactivation of *Cryptosporidium parvum* in demand-free phosphate buffer determined by in vitro excystation and animals infectivity. *Applied and Environmental Microbiology* **59**, 4203–4210.

Fisher AC (1979) Bird migration. *National Geographic* **156**, 154–193.

Garcia LS, Shum AC & Bruckner DA (1992) Evaluation of new monoclonal antibody combination reagent for direct fluorescence detection of *Giardia* cysts and *Cryptosporidium* oocysts in human fecal specimens. *Journal of Clinical Microbiology* **30**, 3255–3257.

Goodgame RW, Genta RM, White AC & Chappbell CL (1993) Intensity of infection in AIDS-associated cryptosporidiosis. *Journal of Infectious Diseases* **167**, 704–709.

Graczyk TK & Cranfield MR (1996) Assessment of the conventional detection of fecal *Cryptosporidium serpentis* oocysts in subclinically infected captive snakes. *Veterinary Research* **27**, 185–192.

Graczyk TK, Cranfield MR & Fayer R (1995) A comparative assessment of direct fluorescence antibody, modified acid-fast-stain, and sucrose flotation techniques for detection of *Cryptosporidium serpentis* in snake fecal specimens. *Journal of Zoo and Wildlife Medicine* **26**, 396–402.

Graczyk TK, Fayer R & Cranfield MR (1996a) *Cryptosporidium parvum* is not cross-transmissible to

fish, amphibia, or reptiles. *Journal of Parasitology* **82**, 748–751.

Graczyk TK, Cranfield MR, Fayer R & Anderson MS (1996b) Viability and infectivity of *Cryptosporidium parvum* oocysts are retained upon intestinal pasage through a refractory avian host. *Applied and Environmental Microbiology* **62**, 3234–3237.

Graczyk TK, Cranfield MR & Fayer R (1996c) Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescence antibody for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum*. *American Journal of Tropial Medicine and Hygiene* **54**, 274–279.

Hansen JS & Ongerth JE (1991) Effect of time and watershed characteristic on the concentration of *Cryptosporidium* oocysts in river water. *Applied and Environmental Microbiology* **57**, 2790–2795.

Hiepe VT & Buchwalder R (1991) Manure as a vector for parasites—a report on experience. *Deutsche Tierärztliche Wochenschrift* **98**, 268–272.

Kilani RT & Sekla L (1987) Purification of *Cryptosporidium* oocysts and sporozoites by cesium chloride and percoll gradients. *American Journal of Tropical Medicine and Hygiene* **36**, 505–508.

Lindsay DS, Blagburn BL & Ernest JA (1987) Experimental *Cryptosporidium* infections in chickens. *Journal of Parasitology* **73**, 242–244.

Lisle JT & Rose JB (1995) *Cryptosporidium* contamination of the water in the USA and UK: a mini-review. *Aqua* **44**, 103–117.

Martin HD & Zeider NS (1992) Concomitant cryptosporidia, coronavirus and parvovirus infection in a raccoon. *Journal of the American Veterinary Medical Association* **181**, 11.

Millard PS, Gensheimer KF & Addiss DG (1994) An outbreak of cryptosporiodiosis from fresh-pressed cider. *Journal of the American Medical Association* **272**, 1592–1596.

Moore AC, Herwaldt BL, Craun GF, Calderon RL, Highsmith AK & Juranek DD (1994) Waterborne diseases in the United States, 1991 to 1992. *Journal of the American Water Works Association* **86**, 87–99.

O'Donoghue PJ (1995) *Cryptosporidium* and cryptosporidiosis in man and animals. *International Journal of Parasitology* **25**, 139–195.

Rose JB, Lisle JT & LeChevallier M (1997) Waterborne cryptosporidiosis: incidence outbreaks and treatment strategies. In *Cryptosporidium and Cryptosporidiosis* (ed R Fayer), CRC Press, Inc., Boca Raton, Florida USA (in press).

Sokal RR & Rohlf FJ (1981) *Biometry*, 2nd edn, W. H. Freeman, New York, New York, USA.

