Rangeland Cattle and the Risk of Waterborne *Cryptosporidium parvum* Infection in Humans

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Background

Cryptosporidium parvum (*C. parvum*) is a tiny protozoal parasite that can cause gastrointestinal illness in a wide variety of mammals, including humans, cattle, sheep, goats, pigs, and horses. It also occurs in various wildlife species such as deer, raccoons, opossums, and rabbits (Fayer *et al.* and Ungar 1990). In cattle, clinical disease and shedding of the parasite is usually limited to calves under a few months of age (National Animal Health Monitoring System 1994, Kirkpatrick 1985, Anderson and Hall 1982). Although not confirmed by studies done in the U.S., researchers in England and in Spain have reported the shedding of *C. parvum* in adult beef cattle (Scott *et al.* 1994, Lorenzo Lorenzo *et al.* 1993). In humans, clinical disease and shedding can appear at all ages, but is typically more common among children (Ungar 1990). The predominant clinical sign is profuse, watery diarrhea lasting from a few days to several weeks in normal (immunocompetent) calves (Kirkpatrick 1985) and humans (Jokipii and Jokipii 1986). While this disease is usually self-limiting in immunocompetent calves and humans, it can be prolonged and life-threatening among immunocompromised people such as AIDS patients. An effective treatment for eliminating this parasite from the gastrointestinal track still does not exist (White *et al.* 1994, Goodgame *et al.* 1993). A few antibiotics may show some promise in reducing the amount of oocyst shedding in AIDS patients, but further clinical trials are needed to fully evaluate their efficacy (White *et al.* 1994, Goodgame *et al.* 1993). The severity of this disease for the immunosuppressed and the fact that this parasite was implicated in recent large scale water-borne outbreaks of gastroenteritis in humans (MacKenzie *et al.* 1994, Hayes *et al.* 1989) has prompted the U.S. Environmental Protection Agency (U.S. EPA), Centers for Disease Control and Prevention, state and local public health agencies and regional water districts to seek ways to reduce surface water contamination of this parasite. Some of this attention has focused on identifying the primary sources of *C. parvum* in surface water. Cattle are often perceived to be a leading environmental source of water-borne *C. parvum*. For example, the U.S. EPA has explicitly warned that inclusion of *C. parvum* into the proposed Enhanced Surface Water Treatment Rule will likely result in new restrictions being placed on the location and management of livestock operations situated within watershed regions (U.S. EPA 1994). Presented below is a brief summary of the medical ecology of *C. parvum* in calves and in humans and the existing scientific evidence that addresses the claim that grazing of cattle on watershed regions puts humans at significant risk for water-borne infection of *C. parvum*.

Life Cycle

In calves and humans, transmission occurs when an infected individual fecally sheds oocysts (eggs) of this parasite into the environment and a susceptible individual inadvertently ingests these oocysts either directly or indirectly through such vectors as contaminated water. The parasite then invades the epithelium of the intestine, replicates, and through sequential reproductive cycles can result, as in the case of calves, in the fecal shedding of up to 10^{10} oocysts per day and up to 10^{7} oocysts per gram of feces (Blewett 1989). Shedding of oocysts can last for 3-12 days in calves (Anderson 1981), allowing for heavy concentrations of oocysts to build up in confined operations. A similar pattern is seen in humans, whereby infected individuals can shed up to 10^{5}-10^{7} oocysts per gram of feces (Goodgame *et al.* 1993), with a duration of shedding varying widely from individual to individual and which can range from a few to more than 50 days (Jokipii and Jokopii 1986). Once shed, these oocysts are immediately infective to another individual, allowing for the rapid spread of this parasite within a group of susceptible individuals.

Oocysts shed from one species of mammal appear to be infectious to other species of mammals. Oocysts from humans have been shown to be infectious to a wide variety of livestock and companion animals (Fayer *et al.* 1990). Oocysts from calves and possibly other mammals appear to be infectious to humans (Dupont *et al.* 1995, Fayer *et al.* 1990). People working with diarrheic calves infected with *C. parvum* have themselves become infected with *C. parvum*, presumably from the calf. However, working with diarrheic calves is not common for the general public.

The Issue

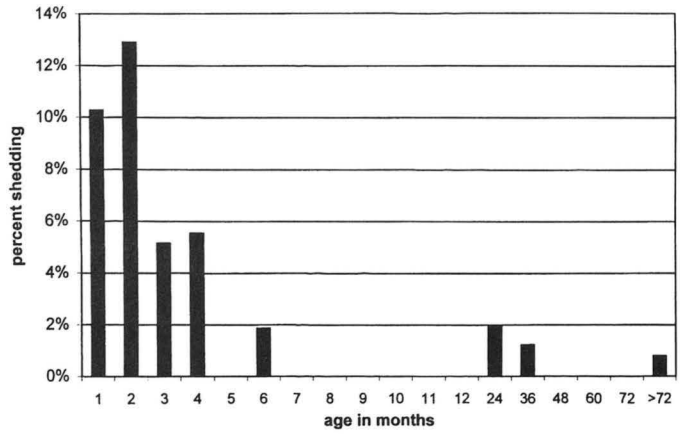
The critical issue is how would *C. parvum* from calves gain access to surface waters and end up in drinking water supplies. The essential steps must include calves becoming infected and shedding the oocysts in their feces. These oocysts must then enter a surface water supply and remain infective as they journey downstream to water treatment plants and distribution systems.

How common is it for calves with access to surface water to shed this parasite? Few studies have been done in beef calves, with most research conducted on dairy calves. Dr. Atwill, University of California-Davis, has been testing cattle from rangeland cow-calf operations from throughout California and has found that most infections are limited to calves under 5 months of age (See Figure 1) (Atwill *et al.*, 1998). One of the largest surveys to date on shedding of *C. parvum* in beef calves was conducted by the USDA's National Animal Health Monitoring System in cooperation with USDA's National Veterinary Services Laboratories. Twenty percent of diarrheic calves (n=391) and 11% of non-diarrheic calves (n=1,053) from a total of 210 operations were found to be shedding *C. parvum* oocysts at the time of sampling (National Animal Health Monitoring System 1994). Around 40% of these 210 operations had one or more calves shedding *C. parvum* oocysts at the time of sampling. In this same study shedding of oocysts was documented in 9% of asymptomatic calves between 3 and 6 months of age, indicating that shedding can occur in these older age groups and without clinical signs. In Manitoba, Canada, 22% of beef calves from 148 herds known to have problems with neonatal diarrhea were found to shed *C. parvum* (Mann *et al.* 1986). In England, 36-39% of diarrheic beef calves tested positive for *C. parvum* while only 8% of healthy beef and dairy calves tested positive (Reynolds *et al.* 1986). From across the United States, 22% of 7,369 dairy calves tested positive for this parasite (Garber *et al.* 1994).

Wildlife Considerations

We are just beginning to study the prevalence of shedding among wildlife species with access to surface waters, but much more research is needed in order to fully understand what contribution wildlife make to surface water contamination. In a survey of 100 raccoons, 13 juveniles had oocysts in their feces (Snyder 1988). Cryptosporidial infection has been confirmed in grey squirrels (Sundberg *et al.* 1982) and in a large variety of neonatal captive deer, including mule deer (Heuschele *et al.* 1986). Thirty percent (35/115) of wild mice trapped at a dairy shed oocysts (Klesius *et al.* 1986). Oocysts obtained from these mice were shown to be infective to calves, perhaps indicating a mouse-calf cycle. Sixty three percent (46/73) of wild brown rats trapped on rural farms were shedding *C. parvum* (Webster and MacDonald, 1995). Atwill *et al.*, 1997, found that 12 (5.4%) of 221 California feral pigs were shedding *C. parvum* oocysts. Younger pigs (≤8 months) and pigs from high density populations (>2.0 feral pigs/km^2) were significantly more likely to shed oocysts compared to older pigs (> 8 months) and pigs from low density populations (≤1.9 feral pigs/km^2). Given the propensity for feral pigs to focus their activity in riparian areas, feral pigs may serve as a source of protozoal contamination for surface water.

Figure 1. Shedding of *Cryptosporidium parvum* by California cow-calf herds.



Human Considerations

The prevalence of shedding among groups of people is highly dependent on which country, which population, and can range from 0-60%, with the higher proportion of shedding among diarrheic individuals (Ungar 1990). The Center for Disease Control and Prevention estimates that the overall background prevalence of shedding in the United States is around 0.5-1.0%, but the relationship between shedding in humans and levels of viable *C. parvum* oocysts in surface water contamination remains unknown. Outbreaks of human cryptosporidiosis have been linked to swimming in pools (Bell *et al.* 1993). Also unknown is what proportion of cryptosporidiosis in humans is due to water-borne infection as opposed to human-to-human direct infection.

Survivability

How long do *C. parvum* oocysts survive in the environment once they are shed in feces? Oocysts became non-viable after several hours of in-door drying at room temperature (Robertson *et al.* 1992). Oocysts recovered from calf fecal patties which had been kept inside a barn (summer) or inside an unheated shed (winter) became non-infective for 3-7 day old mice in 1-4 days (Anderson 1986). If fecal material thoroughly dries before reaching water, the oocysts would presumably become non-infectious for animals and humans. Ten or more days of freezing at -22°C caused over 90% of oocysts to become non-viable (Robertson *et al.* 1992). Using mice to determine infectivity, as few as 24 hours of freezing at -20°C or as few as 7 days of freezing at -15°C appeared to render the oocysts non-infective (Fayer and Nerad, 1996). This would suggest that oocysts shed by calves during winter conditions may not survive through the season. Oocysts in distilled water became non-infective if heated to 72.4°C or higher for 1 minute or if heated to 64.2°C or higher for 2 minutes (Fayer 1994). What if fecal material is deposited directly in a stream? One study found that after 33 days in river water, an estimated 40-44% of purified oocysts were incapable of excystation. After 176 days, 89-99% were estimated to be incapable of excystation (Robertson *et al.* 1992). Approximately 67-88% of oocysts were still viable after 33 days when kept in manure at 4°C (Jenkins *et al.*, 1997; Robertson *et al.* 1992). Environmental survival of *C. parvum* is an area of active research and we should have considerably more information in the next few years regarding how quickly oocysts die in our western climates.

It may be that most oocysts do not remain infective as they journey from infected calves to surface water to water treatment plant to human consumption. Although there are severe environmental pressures for oocysts to remain infective when excreted on land, apparently only a few oocysts would need to remain viable in order to pose a risk to humans. Experimental studies in healthy humans determined that the infectious dose at which 50% of subjects acquire infection (ID_{50}) was 132 calf-derived oocysts, with as few as 30 oocysts sufficient to induce cryptosporidiosis (Dupont *et al.* 1995).

Cattle and Cryptosporidium in Water

What evidence directly links the presence of *C. parvum* in surface water supplies to livestock production? In attempting to answer this question, one must test samples of water which is a procedure with some limitations. Environmental studies to date have had a difficult time determining if the Cryptosporidium found in surface water is *C. parvum* or some other Cryptosporidium species not infectious to humans, potentially not shed by cattle, yet detected by one of the laboratory assays used for environmental testing. For example, it appears that *C. muris* which is shed by cattle, rodents, and other mammals, *C. meleagridis* which is shed by turkeys, *C. serpentis* which is shed by snakes, and various other isolates of Cryptosporidium found in lizards can cross-react to some degree with the Merifluor monoclonal antibody produced by Meridian Diagnostics, Inc. (Graczyk *et al.*, 1996; Smith and Rose 1990). All of these species of Cryptosporidium are not known to be infectious to humans. Dr. Atwill has confirmed in his laboratory that isolates of *C. muris* obtained from adult dairy cattle from the central San Joaquin Valley in California can cross react with the Merifluor monoclonal antibody. Hence, the possibility of false positives is very likely when testing water for *C. parvum*. On the other hand, the recovery efficiency for testing raw water samples, which is the estimated proportion of oocysts recovered out of all oocysts initially present, can vary from below 10% to as high as 60% (Smith and Rose 1990). Hence, water samples with lower concentrations of oocysts could be erroneously classified as negative. With these limitations in mind (likelihood of false positives or false negatives), *C. parvum* oocysts are quite common throughout the surface water supplies of the United States. For example, 50-60% of raw water samples from primarily Midwest and East Coast surface water sources were positive for Cryptosporidium oocysts. The geometric mean of detectable levels of Cryptosporidium oocysts was 2.4-2.7 oocysts/Liter, with a range between 0.07 and 484 oocysts/Liter (LeChevallier and Norton 1995, LeChevallier *et al.* 1991). Rose, 1988, detected Cryptosporidium oocysts in 51% of 111 raw surface water samples from 13 states. Large west coast rivers in Washington and in California were found to have concentration of Cryptosporidium oocysts ranging from 2 to 112 oocysts/Liter, with a mean of 25 oocysts/Liter (Ongerth and Stibbs 1987). Yet, the link between beef cattle grazing and elevated levels of Cryptosporidium oocysts in surface water is not very clear. For example, one study found little difference in the concentration of Cryptosporidium oocysts from protected surface waters (0.3-4.0 oocysts/Liter) as compared to surface waters subject to agricultural run-off (0.1-2.0 oocysts/Liter) (LeChevallier *et al.* 1991). Moreover, 68% of these oocysts had become non-viable. Another study measured 5,800 oocysts/Liter in irrigation canal water running through agricultural acreage with cattle pastures (beef or dairy not specified), compared to 127 oocysts/Liter in river water subject to human recreation and 0.8 oocysts/Liter for stream water exposed to ranch land runoff (Madore *et al.* 1987). One of the most compelling studies to date was conducted on grazed watersheds in British Columbia. They found concentrations of Cryptosporidium oocysts in river water to be higher just

below a cattle ranch (13.3 oocysts/100 Liters) compared to the samples just above the ranch (5.6 oocysts/100 Liters) (Ong *et al.*, 1996). These differences appeared to be limited to the period when young calves were present on the watershed and presumably rainfall events were occurring (spring). It would be interesting to know whether increases of waterborne Cryptosporidium would have occurred in the absence of young calves or rainfall. Unfortunately, the species of Cryptosporidium was not specified, but was most likely *C. parvum* since the increase in waterborne Cryptosporidium was most dramatic during and just following the calving season. Cryptosporidium oocysts from pristine surface waters have been found to contain 0.005-18 oocysts/Liter, indicating that this organism occurs naturally in pristine watersheds (Madore *et al.* 1987). This would suggest that wildlife need to be carefully examined for their role in contaminating surface water with this parasite.

Diagnostic Procedures For Fecal Samples

Sensitive and specific procedures are needed for rapidly diagnosing clinical cryptosporidiosis in humans and animals and for detecting *C. parvum* in environmental samples which may serve as reservoirs of infection. A variety of antemortem procedures are available for diagnosing *C. parvum* infections in mammals. The majority of procedures are designed to detect oocysts in human or animal fecal samples. Direct smears of diluted fecal samples is a very basic procedure, but suffers from low sensitivity and the necessity of differentiating yeast and other organisms from oocysts (MacPherson and McQueen 1993). Concentration procedures, such as sedimentation using formalin-ethyl acetate (Weber *et al.* 1992) or flotation over sucrose (Current 1990) or salt solutions such as sodium chloride (Weber *et al.* 1992) or sodium dichromate (Johnson *et al.* 1997), can increase the sensitivity of the test (probability of detecting an infected individual given that it is infected), yet one must be able to differentiate a variety of microscopic particulate matter from oocysts (for a review see Arrowood 1997 and Current 1990). Various stains have been developed to improve the specificity and sensitivity of detecting *C. parvum* oocysts in dried fecal smears. Common stains include Ziehl-Neelsen (Heriksen and Pohlenz 1981), Kinyoun, and auramine and rhodamine (MacPherson and McQueen 1993). While the Ziehl-Neelsen acid fast procedure requires only a light microscope, the auramine and rhodamine procedure requires fluorescent microscopy. It should be kept in mind that some stains will cross-react with particulate matter in the fecal sample such as some yeasts, creating the possibility of false positives.

A variety of monoclonal-based procedures have been developed for detecting *C. parvum* oocysts in fecal samples. A direct immunofluorescent antibody test (Merifluor Cryptosporidium/ Giardia) has been evaluated and does not cross-react with non-Cryptosporidium protozoa, helminths egg and larvae, and various bacteria and yeast commonly found in human stool samples (Garcia *et al.* 1992), but a rigorous evaluation has not been performed for the diversity of microorganisms found in domestic animal manure. Using human fecal samples, this direct immunofluorescent antibody test was shown to reliably detect down to 5,000 oocysts per gram of watery stool and 50,000 oocysts per gram of formed stool (Weber *et al.* 1991). We have found in our laboratory that this test can reliably detect 1000 oocysts per gram of ruminal bovine manure (unpublished data). Commercially available enzyme-linked immunosorbent assays are now available which have a similar sensitivity to direct immunofluorescence and which can eliminate the time consuming task of microscopic examination of each fecal sample (Rosenblatt and Sloan 1993). Subsequent work has shown that commercially available direct immunofluorescent assays and enzyme-linked immunosorbent assays cross-react with other species of Cryptosporidium, such as isolates of *C. muris, C. wrairi, C. meleagridis,* and *C. serpentis* (Graczyk *et al.* 1996). A detailed review of diagnostic procedures for foals can be found in *The Compendium*, 1996, Vol 18: 298-306.

Molecular methods of detecting *C. parvum* oocysts are under rapid development with a growing number of diagnostic primers being developed for the sensitive and specific detection of this parasite in stool or manure samples. These methods have the potential to detect very low numbers of oocysts. Several methods under development are reviewed in Arrowood 1997.

Conclusion

Until we have more detailed studies which provide a causal link between grazing practices and elevated levels of infective *C. parvum* in nearby surface water across different watersheds, different ranching operations and across different seasons, it would be premature to claim that cattle production is a leading environmental source of infective *C. parvum* for water. The presence of young calves in close proximity to surface water during conditions of rainfall may lead to *C. parvum* contamination of surface water, but these conditions (young calves and rain) are not present year round on our western watersheds. Instead, much of our private and public grazing land enjoy only seasonal rain and calving seasons are in many cases not coincident with this rainfall season. As such, contaminating water with bovine *C. parvum* would be very difficult when young calves are not present or when rainfall is not occurring, unless of course young calves are allowed to

defecate directly into streams, rivers, reservoirs or lakes. Lastly, if in the words of US EPA (1994) we are to *"minimize the potential for source water contamination"* by *C. parvum*, then we must first identify the primary quantitative source(s) of this parasite in the environment, be it livestock, wildlife, humans, companion animals, or human-associated sewage effluent, and continue to unravel the medical ecology of this parasite.

References

Anderson, B.C. 1981. Patterns of shedding of cryptosporidial oocysts in Idaho calves. J. Am. Vet. Med. Assoc. 178:982-984. Anderson, B.C. and R.F. Hall. 1982. Cryptosporidial infection in Idaho dairy calves. J. Am. Vet. Med. Assoc. 181:484-485. Anderson, B.C. 1986. Effect of drying on the infectivity of cryptosporidia-laden calf feces for 3- to 7-day old mice. Am. J. Vet. Res. 47:2272-2273. Arrowood, M.J. 1997. Diagnosis, p. 43-64. In: R. Fayer (ed.), Cryptosporidium and Cryptosporidiosis. CRC Press, Boca Raton. Atwill, E.R., R.A. Sweitzer, M. Das GraÁas C. Pereira, *et al.* 1997. Prevalence of and associated risk factors for shedding Cryptosporidium parvum and Giardia within feral pig populations in California. Appl. Environ. Microbiol. 63: 3946-3949. Atwill, E.R., E. Johnson, D.J. Klingborg, *et al.* 1998. Distribution of fecal shedding of Cryptosporidium parvum oocysts in cow-calf herds in California. Am. J. Vet. Res. (under review). Bell, A., R. Guasparini, D. Meeds, *et al.* 1993. A swimming pool-associated outbreak of cryptosporidiosis in British Columbia. Can. J. Public .Health. 84:344-7. Blewett, D.A. 1989. Quantitative techniques in Cryptosporidium research, p. 85-95. In: K.W. Angus and D.A. Blewett (eds.), Proc. 1st Int. Workshop. Cryptosporidiosis Edinburgh, Scotland. Current, W.L. 1990. Techniques and laboratory maintenance of Cryptosporidium, p. 31-50. In: J.P. Dubey, C.A. Speer, and R. Fayer (eds.), Cryptosporidiosis of man and animals. CRC Press, Boca Raton. DuPont, H.L., C.L. Chappell, C.R. Sterling, *et al.* 1995. The infectivity of Cryptosporidium parvum in healthy volunteers. N. Engl. J. Med. 332:855-859. Fayer, R. and T. Nerad. Effects of low temperatures on viability of Cryptosporidium parvum oocysts. Appl. Environ. Microbiol. 62:1431-33. Fayer, R. 1994. Effect of high temperature on infectivity of Cryptosporidium parvum oocysts in water. Appl. Environ. Microbiol. 60:2732-35. Fayer, R., C.A. Speer and J.P. Dubey. 1990. General biology of Cryptosporidium, p. 2-29. In: J.P. Dubey, C.A. Speer and R. Fayer (eds.), Cryptosporidiosis of man and animals. CRC Press, Boca Raton, Fla. Garber, L.P., M.D. Salman, H.S. Hurd, *et al.* 1994. Potential risk factors for Cryptosporidium infection in dairy calves. J. Am. Vet. Med. Assoc. 205:86-91. Goodgame, R.W., R.M. Genta, A.C. White, *et al.* 1993. Intensity of infection in AIDS-associated cryptosporidiosis. J. Inf. Dis. 167:704-709. Garcia, L.S., A.C. Shum, and D.A. Bruckner. Evaluation of a new monoclonal antibody combination reagent for direct fluorescence detection of Giardia cysts and Cryptosporidium oocysts in human fecal samples. J. Clin. Microbiol. 30:3255-3257. Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1996. Evaluation of commercial immunoassay (EIA) and immunofluorescent antibody (IFA) test kits for detection of Cryptosporidium oocysts of species other than Cryptosporidium parvum. Am. J. Trop. Med. Hyg. 54:274-279. Hayes, E.B., T.D. Matte, T.R. O'Brien, *et al.* 1989. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. N. Engl. J. Med. 320:1372-1376. Henriksen, S. and J. Pohlenz. 1981. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand. 22:594-596. Heuschele, W.P., J. Oosterhuis, D. Janssen, *et al.* 1986. Cryptosporidial infections in captive wild animals. J. Wild. Dis. 22:493-495. Kirkpatrick, C.E. 1985. Cryptosporidium infection as a cause of calf diarrhoea. Vet. Clin. North Am. Food Anim. Pract. 1:515:528. Klesius, P.H., T.B. Hayes and L.K. Malo. 1986. Infectivity of Cryptosporidium sp isolated from wild mice for calves and mice. J. Am. Vet. Med. Assoc. 189:192-193. Jenkins, M.B., L.J. Anguish, D.D. Bowman, *et al.* 1997. Assessment of a dye permeability assay for determination of inactivation rates of Cryptosporidium parvum oocysts. Appl. Environ. Microbiol. 63:3844-50. Jokipii, L. and M.M. Jokipii. 1986. Timing of symptoms and oocyst excretion in human cryptosporidiosis. N. Engl. J. Med. 315:1643-46. Johnson, E., E.R. Atwill, M.E. Filkins, and J. Kalush. 1997. The prevalence of shedding of Cryptosporidium and Giardia spp. based on a single fecal sample collection from each of 91 horses used for backcountry recreation. J. Vet. Diagn. Invest. 9:56-60. LeChevallier, M.W., W.D. Norton and R.G. Lee. 1991. Occurrence of Giardia and Cryptosporidium spp. in surface water supplies. Appl. Environ. Microbiol. 57:2610-16. LeChevallier, M.W. and W.D. Norton. 1995. Giardia and Cryptosporidium in raw and finished water. J. Am. Water. Works. Assoc. 87:54-68. Lorenzo Lorenzo, M.J., E. Ares-Maz·s and I. Villacorta Martinez de Maturana. 1993. Detection of oocysts and IgG antibodies to Cryptosporidium parvum in asymptomatic adult cattle. Vet. Parasitol. 47: 9-15. MacKenzie, W.R., N.J. Hoxie, M.E. Proctor, *et al.* 1994. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. N. Engl. J. Med. 331:161-167. Madore, M.S., J.B. Rose, C.P. Gerba, *et al.* 1987. Occurrence of Cryptosporidium oocysts in sewage effluents and selected surface waters. J. Parasit. 73:702-5. Mann, E.D., L.H. Sekla, G.P.S. Nayar, *et al.* 1986. Infection with Cryptosporidium spp. in humans and cattle in Manitoba. Can. J. Vet. Res. 50:174-8. MacPherson, D.W., and R. McQueen. 1993. Cryptosporidiosis: multiattribute evaluation of six diagnostic methods. J. Clin. Microbiol. 31:198-202. National Animal Health Monitoring System Staff. 1994. Cryptosporidium and Giardia in beef calves. VS, APHIS, USDA, Fort Collins, Colo. Ong, C., W. Moorehead, A. Ross, and J. Isaac-Renton. 1996. Studies of Giardia spp. and Cryptosporidium spp. in two adjacent watersheds. Appl. Environ. Microbiol. 62:2798-2805. Ongerth, J.E. and H.H. Stibbs. 1987. Identification of Cryptosporidium oocysts in river water. Appl. Environ. Microbiol. 53:672-6. Reynolds, D.J., J.H. Morgan, N. Chanter, *et al.* 1986. Microbiology of calf diarrhoea in southern Britain. Vet. Rec. 119:34-9. Robertson, L.J., A.T. Campbell and H.V. Smith. 1992. Survival of Cryptosporidium parvum oocysts under various environmental pressures. Appl. Environ. Microbiol. 58:3494-3500. Rose, J. 1988 Occurrence and significance of Cryptosporidium in water. J. Am. Water. Works. Assoc. 2:53-58. Rosenblatt, J.E., and L.M. Sloan. 1993. Evaluation of an enzyme-linked immunosorbent assay for detection of Cryptosporidium spp. in stool specimens. J. Clin. Microbiol. 31:1468-1471. Scott, C.A., H.V. Smith and H.A. Gibbs. 1994. Excretion of Cryptosporidium parvum oocysts by a herd of beef suckler cows. Vet. Rec. 134:172. Smith, H.V. and J.B. Rose. 1990. Waterborne cryptosporidiosis. Parasitol. Today 6:8-12. Snyder, D.E. 1988. Indirect immunofluorescent detection of oocysts of Cryptosporidium parvum in the feces of naturally infected racoons (Procyon lotor). J. Parasitol . 74:1050-55. Sundberg, J.P., D. Hill D and M.J. Ryan. 1982. Cryptosporidiosis in a gray squirrel. J. Am. Vet. Med. Assoc. 181:1401-2. Ungar, B.L.P. 1990. Cryptosporidiosis in humans (Homo sapiens), p. 59-82. In: J.P. Dubey, C.A. Speer and R. Fayer (eds.), Cryptosporidiosis of man and animals. CRC Press, Boca Raton, Fla. U.S. EPA. 1994. National Primary Drinking Water Regulations: Enhanced Surface Water Treatment Requirements. Fed. Register 59:38832-38858. Weber, R., R.T. Bryan, H.S. Bishop, S.P. Wahlquist, J.L. Sullivan, and D.D. Juranek. 1991. Threshold of detection of Cryptosporidium oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. J. Clin. Microbiol. 29:1323-1327. Weber, R., R.T. Bryan, and D.D. Juranek. 1992. Improved stool concentration procedure for detection of Cryptosporidium oocysts in fecal specimens. J. Clin. Microbiol. 30:2869-2873. Webster, J.P. and D.W. MacDonald. Cryptosporidiosis reservoir in wild brown rates (Rattus norvegicus) in the UK. Epidemiol. Infect. 115:207-209. White, A.C., C.I. Chappell, C.S. Haya, *et al.* 1994. Paromycin for cryptosporidiosis in AIDS: a prospective, double-blind trial. J. Infect. Dis. 70:419-24.