Treatment and prevention of cryptosporidiosis: what options are there for a country like Zambia?

PAUL KELLY*

Barts and The London School of Medicine, Queen Mary University of London, London; Tropical Gastroenterology and Nutrition group, University of Zambia School of Medicine, Lusaka, Zambia

(*Received 27 September 2010; revised 20 December 2010; accepted 21 December 2010; first published online 15 February 2011*)

SUMMARY

Cryptosporidiosis is a major infection of humans, leading to diarrhoea and growth failure in children, diarrhoea and malnutrition in immunocompromised adults, and is associated with increased mortality in all age groups. Using the country of Zambia as an example, I review the possible approaches to treatment and prevention in a tropical setting. The current optimal therapy for cryptosporidiosis is nitazoxanide which works well in HIV uninfected children, but treatment in patients with HIV infection remains remarkably difficult. No single drug has demonstrated efficacy in a randomised trial. No vaccine is available, so the best option for prevention for the moment is filtration and clean storage of drinking water. This would be expected to reduce cryptosporidiosis dramatically, but this needs to be demonstrated directly. Water filtration would have the added benefit of protection against many other pathogens, but the paucity of alternative approaches highlights the need for a better understanding of this important human pathogen.

Key words: Cryptosporidiosis, *Cryptosporidium*, vaccine, diarrhoeal disease, nitazoxanide, paromomycin, Africa, water filtration.

INTRODUCTION

My intention is to give a rather personal perspective on the prospects for being able to treat or control cryptosporidiosis. I have chosen to use Zambia as an example as I have worked in Zambia for over 20 years, and a good deal of the work I have undertaken has been on cryptosporidiosis in children and in adults. While Zambia will have a great deal in common with other tropical countries, there may also be substantial differences. For example, while cryptosporidiosis is a major contributor to morbidity and mortality in malnourished children in Lusaka, this appears less true in Malawi, Zambia's immediate neighbour to the east. So while much of what we have learned about cryptosporidiosis in Zambia may be true in other parts of Africa, some of it may be more geographically restricted.

WHAT IS THE PROBLEM?

Of all the 19 species in the genus *Cryptosporidium* (Fayer, 2010) there are only two which commonly infect man: *C. parvum* and the more recently described *C. hominis*. There are occasional reports of infections with other species, including *C. meleagridis*, *C. felis* and *C. canis* (for example see Lucca *et al.* 2009), and there is evidence from Nigeria of a high degree of species diversity during infection, including *C. meleagridis*, *C. canis* and unclassified rabbit and cervine genotypes (Molloy *et al.* 2010). *C. hominis* is restricted to human hosts whereas *C. parvum* has a zoonotic reservoir. The literature suggests that there is a higher diversity of *Cryptosporidium* species in human infections in tropical areas (for example see Adamu *et al.* 2010; Molloy *et al.* 2010). In a recent study of dairy farms in Zambia, 34% of 207 dairy calves were shedding *Cryptosporidium* oocyst antigens (as assessed by ELISA), 10% of dairy workers were shedding antigens, and 5% of household contacts were shedding antigens (Siwila *et al.* 2007). The transmission of cryptosporidiosis is evidently on a large scale in rural Africa. In a study of children with diarrhoea in one community in Lusaka, *Cryptosporidium* oocysts were found in 18% of 222 episodes (Nchito *et al.* 1998), thus confirming that cryptosporidiosis is also an urban problem.

Worldwide the picture is the same. Cryptosporidiosis is a major contributor to diarrhoeal disease in children (Mor and Tzipori, 2008). Importantly, among the diarrhoea-causing pathogens, cryptosporidiosis is associated with worse outcomes. Mortality was higher among children with cryptosporidiosis in Guinea-Bissau (Molbak *et al.* 1993), and was associated with impaired subsequent development in studies in Guinea-Bissau and in Brazil (Molbak *et al.* 1997; Newman *et al.* 1999). In Zambia too, cryptosporidiosis in hospitalised malnourished

* Corresponding author: Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine, Queen Mary University of London, Turner Street, London E1 2AD. Tel: 020 7882 2643. Fax: 020 7882 7192. E-mail: m.p.kelly@qmul.ac.uk

doi:10.1017/S0031182011000035

children was associated with higher mortality (Amadi *et al*. 2001). There can be no doubt that treatment and prevention of cryptosporidiosis would be highly desirable.

TREATMENT OF CRYPTOSPORIDIOSIS

The current standard of care for treatment of cryptosporidiosis in children is nitazoxanide (Pantenburg *et al*. 2009). This thiazolide has a wide spectrum of antimicrobial activity, including helminths, protozoa and viruses (notably hepatitis C virus; Rossignol, 2009). Given to children in a dose of 100 mg twice daily for 3 days it hastens clinical remission, increases parasitological clearance and, in at least one study in Zambia, reduced mortality (Amadi *et al*. 2002). However, in this study from Zambia it was apparent that nitazoxanide was not effective in children with HIV infection, as no effect was seen against any endpoint. We have subsequently gone on to examine the possible effect of higher dose (200 mg twice daily) and prolonged treatment (28 days) in HIV-infected children, but still there was no effect (Amadi *et al*. 2009).

This lack of effect in cryptosporidiosis in children with AIDS is sadly consonant with the difficulty in finding any effective treatment in adults with AIDS, a long history of an unrewarding search for an effective therapy. Over 100 agents have been tried, without success. The most promising agent before nitazoxanide was paromomycin, but this too has not shown efficacy when evaluated formally (Hewitt *et al*. 2000). Recent systematic reviews conclude that there is no effective treatment for cryptosporidiosis in immunocompromised patients (Abubakar *et al*. 2007). We have recently completed a phase 1 evaluation of the potential of miltefosine in cryptosporidiosis in AIDS patients. Seven adults with AIDS-related cryptosporidiosis or related infections were evaluated, but safety could not be confirmed and the trial was abandoned (Sinkala *et al*. 2011). It would appear that the difficulty in maintaining good hydration in patients with severe diarrhoeal disease precipitates renal toxicity, but a high frequency of hepatic dysfunction was also observed.

There were attempts in the 1980s and 1990s to treat cryptosporidiosis with passive immunotherapy (Nord *et al*. 1990). Hyperimmune bovine colostrum was given orally as a source of antibodies to several stages of the life cycle, but this form of therapy was never subjected to a randomised controlled trial and its use in a resource-limited setting would be unlikely even if it did work.

Nitazoxanide and miltefosine would both have the merit of being affordable in any country where health budgets are severely limited. Obviously this is not true of passive immunotherapy. What are the prospects of new, affordable antiprotozoal drugs? In a recent analysis of novel thiazolide anti-protozoal agents (Gargala *et al*. 2010) there may be some promising compounds but much further evaluation is needed. However, the current position is that new approaches are probably needed and combination therapy is likely among them.

PREVENTION OF CRYPTOSPORIDIOSIS

There are no vaccines against any species in the genus *Cryptosporidium*; indeed there are no commercially available vaccines against any human parasitosis. Immunisation against protozoa is not intrinsically impossible, as there has been a highly successful vaccine against *Eimeria tenella* in veterinary use for many years (McDonald and Shirley, 2009). However, immunisation against *Plasmodium* (which is closely related to *Cryptosporidium*) has so far been elusive and without question malaria is a much more important human disease than cryptosporidiosis in terms of the overall burden of morbidity and mortality. With so much less funding available for research into cryptosporidiosis than for malaria, the prospects of a vaccine are indeed remote. So without a vaccine, we have to examine the prospects for interruption of transmission.

Water-borne transmission is probably a major contributor to the spread of infection. In any given endemic disease setting the relative contributions of water-borne, zoonotic and direct person-to-person transmission to the overall burden of cryptosporidiosis are hard to quantify, but there is no doubt that water-borne transmission is the source of several major epidemics and probably a very large number of minor epidemics. The largest epidemic on record affected Milwaukee in 1993 with over 400,000 cases (McKenzie *et al*. 1994). It is distinctly possible that 'endemic' infection in fact comprises a series of overlapping micro-epidemics, to which water-borne transmission would make a significant contribution. In Lusaka, we identified oocyst contamination of the municipal water supply and were able to detect a correlation between the intensity of oocyst contamination and the prevalence of cryptosporidiosis in the townships supplied from that water source (Kelly *et al*. 1997). The contamination of the water supply showed seasonal variation (Fig. 1) which correlated with seasonal infection incidence in this and subsequent studies (Kelly *et al*. 2009). Our estimate of the population attributable risk fraction of cryptosporidiosis attributable to the presence of water-borne oocysts was 25%, which although a very crude (and probably under-) estimate would suggest that interventions to interrupt water-borne transmission would reduce overall disease burden considerably.

As noted above, zoonotic transmission is likely also to contribute to the disease burden in a country like Zambia. Farm workers can acquire the infection directly and then transmit it directly to other members of their households, or they may acquire

https://doi.org/10.1017/S0031182011000035 Published online by Cambridge University Press

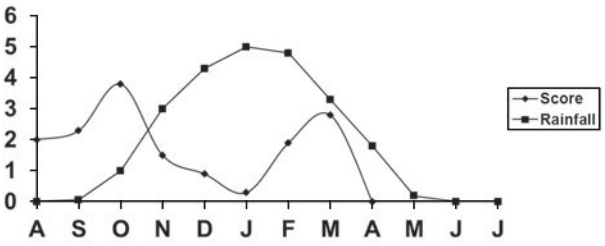

Fig. 1. Score of intensity of oocyst contamination of principal (river-drawn) municipal water inflow to Lusaka during 1995–1997 set alongside rainfall averaged over these years. Oocyst contamination was measured using DPPPY MicroWynd filters (Cuno Europe, Tachbrook Park, Warwick, UK) which were used to sample from 100 litres of water drawn at the water treatment plant inflow. A series of smears was made from the washes of the filters and expressed as a score using arbitrary units as previously described (Kelly *et al*. 1997). Rainfall is the monthly Lusaka average (× 25 mm to allow visualisation on the same scale) over the period 1991–1996 (data from Zambia meteorological office, Lusaka).

infection from shared, contaminated drinking water. Cattle are major reservoirs of infection, which contaminate surface waters during periods of rainfall. One would expect that the ratio of *C. parvum* to *C. hominis* would be considerably greater in rural than in urban populations, but this has not been tested directly. As either species could contaminate drinking water, and either species can be transmitted directly person-to-person, only formal trials of interventions will truly determine the dominant routes of transmission and simultaneously inform public health policy.

What interventions need to be evaluated? First, filtration technologies have been reduced in scale such that cheap domestic units are available (such as the Life Straw, Vestergard Frandsen, Switzerland, Fig. 2). Second, water boiling is effective against *Cryptosporidium* oocysts, but given the expense of fuel it is not clear how effective this intervention would be at a population level. My own experience is that residents of Zambian peri-urban 'compounds' use boiling to disinfect drinking water only when there is a public health emergency such as a cholera epidemic. Very few households do this every day. Third, ultraviolet sterilisation has been reported to have significant impact on childhood diarrhoeal disease, and SOlar water DISinfection (SODIS) is being rolled out at scale, but to my knowledge its impact on cryptosporidiosis has not been studied. It would be predicted to be effective if water turbidity is not too high and post-treatment contamination is not too intense. Fourth, intensive handwashing has been reported to reduce diarrhoeal disease, including cryptosporidiosis, in American AIDS patients (Huang and Zhou, 2007) but this finding has not to our knowledge been replicated in a tropical setting.

Fig. 2. Domestic water filtration device (Life Straw, Vestergard Frandsen) in use in Lusaka. This filtration unit contains a pre-filter to remove larger particulates and a textile filter to remove microbe contaminants with an effective pore size of 20 nm. The unit is expected to last for 3 years with a daily load of 16 litres.

CONCLUDING REMARKS

The prospects for control of cryptosporidiosis for a sub-Saharan African country like Zambia are not promising in the short term. The most immediately productive approach would be completion of formal controlled trials of interventions based on filtration and clean storage of drinking water. Such interventions would be predicted to have a very considerable impact not only on cryptosporidiosis but on a wide range of diarrhoea-causing pathogens. Hand washing interventions may also help. As diarrhoea remains one of the major causes of the still unacceptably high mortality in children under 5 years of age, filtration interventions remain a very attractive avenue to pursue. Effective treatment remains further off, even though those of us who are physicians feel the urgency of the need to be able to offer more effective treatments to our patients than we can at the moment.

FINANCIAL SUPPORT

Much of our work described was supported by the Wellcome Trust and Romark Laboratories.

CONFLICT OF INTEREST

I am co-investigator in a clinical trial funded by Vestergard Frandsen.

REFERENCES

Abubakar, I., Aliyu, S. H., Arumugam, C., Hunter, P. R. and Usman, N. K. (2007). Prevention and treatment of cryptosporidiosis in immunocompromised patients. *Cochrane Database of Systematic Reviews* **24**, CD004932.

Adamu, H., Petros, B., Hailu, A. and Petry, F. (2010). Molecular characterisation of *Cryptosporidium* isolates from humans in Ethiopia. *Acta Tropica* **115**, 77–83.

Amadi, B. C., Kelly, P., Mwiya, M., Mulwazi, E., Sianongo, S., Changwe, F., Thomson, M., Hachungula, J., Watuka, A., Walker-Smith, J. A. and Chintu, C. (2001). Intestinal and systemic infection, HIV and mortality in Zambian children with persistent diarrhoea and malnutrition. *Journal of Pediatric Gastroenterology and Nutrition* **32**, 550–554.

Amadi, B. C., Mwiya, M., Musuku, J., Watuka, A., Sianongo, S., Ayoub, A. and Kelly, P. (2002). Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. *Lancet* **360**, 1375–1380.

Amadi, B., Mwiya, M., Sianongo, S., Payne, L., Watuka, A., Katubulushi, M. and Kelly, P. (2009). High dose prolonged treatment with nitazoxanide is not effective for cryptosporidiosis in HIV positive Zambian children: a randomised controlled trial. *BMC Infectious Diseases* **9**, 195.

Fayer, R. (2010). Taxonomy and species delimitation in *Cryptosporidium*. *Experimental Parasitology* **124**, 90–97.

Gargala, G., Le Goff, L., Ballet, J.-J., Favennec, L., Stachulski, A. V. and Rossignol, J. F. (2010). Evaluation of new thiazolide/thiadiazolide derivatives reveals nitro group-independent efficacy against *in vitro* development of *Cryptosporidium parvum*. *Antimicrobial Agents and Chemotherapy* **54**, 1315–1318.

Hewitt, R. G., Yiannoutsos, C. T., Higgs, E. S., Carey, J. T., Geiseler, P. J., Soave, R., Rosenberg, R., Vazquez, G. J., Wheat, L. J., Fass, R. J., Antoninievic, Z., Walawander, A. L., Flanigan, T. P. and Bender, J. F. (2000). Paromomycin: no more effective than placebo for the treatment of cryptosporidiosis in patients with advanced HIV infection. AIDS Clinical Trial Group. *Clinical Infectious Disease* **31**, 1084–1092.

Huang, D. B. and Zhou, J. (2007). Effect of intensive handwashing in the prevention of diarrhoeal illness among patients with AIDS: a randomized controlled study. *Journal of Medical Microbiology* **56**, 659–663.

Kelly, P., Nchito, M., Baboo, K. S., Ndubani, P., Okeowo, N. P., Luo, N. P., Feldman, R. A. and Farthing, M. J. G. (1997). Cryptosporidiosis in adults in Lusaka, Zambia, and its relationship to oocyst contamination of drinking water. *Journal of Infectious Diseases* **176**, 1120–1123.

Kelly, P., Todd, J., Sianongo, S., Mwansa, J. C. L., Sinsungwe, H., Katubulushi, M., Farthing, M. J. G. and Feldman, R. A. F. (2009). Susceptibility to intestinal infection and diarrhoea in Zambian adults in relation to HIV status and CD4 count. *BMC Gastroenterology* **9**, 7.

Lucca, P., De Gaspari, E. N., Bozzoli, L. M., Funada, M. R., Silva, S. O., Iuliano, W. and Soares, R. M. (2009). Molecular characterization of *Cryptosporidium* spp. from HIV infected patients from an urban area of Brazil. *Revista Instituto Medica Tropica Sao Paulo* **51**, 341–343.

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. and Rose, J. B. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine* **331**, 161–167.

McDonald, V. and Shirley, M. W. (2009). Past and future: vaccination against Eimeria. *Parasitology* **136**, 1477–1489.

Molloy, S. F., Smith, H. V., Kirwan, P., Nichols, R. A., Asaolu, S. O., Connelly, L. and Holland, C. V. (2010). Identification of a high diversity of *Cryptosporidium* species genotypes and subtypes in a pediatric population in Nigeria. *American Journal of Tropical Medicine and Hygiene* **82**, 608–613.

Molbak, K., Andersen, M., Aaby, P., Højlyng, N., Jakobsen, M., Sodemann, M. and da Silva, A. P. (1997). *Cryptosporidium* infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, West Africa. *American Journal of Clinical Nutrition* **65**, 149–152.

Molbak, K., Hojlyng, N., Gottschau, A., Sá, J. C., Ingholt, L., da Silva, A. P. and Aaby, P. (1993). Cryptosporidiosis in infancy and childhood mortality in Guinea Bissau. *British Medical Journal* **307**, 417–420.

Mor, S. M. and Tzipori, S. (2008). Cryptosporidiosis in children in sub-Saharan Africa: a lingering challenge. *Clinical Infectious Diseases* **47**, 915–921.

Nchito, M., Kelly, P., Sianongo, S., Luo, N. P., Feldman, R. A., Farthing, M. J. G. and Baboo, K. S. (1998). Cryptosporidiosis in urban Zambian children: an analysis of risk factors. *American Journal of Tropical Medicine and Hygiene* **59**, 435–437.

Newman, R. F. D., Sears, C. L., Moore, S. R., Nataro, J. P., Wuhib, T., Agnew, D. A., Guerraut, R. L. and Lima, A. A. (1999). Longitudinal study of *Cryptosporidium* infection in children in Northeastern Brazil. *Journal of Infectious Diseases* **180**, 167–175.

Nord, J., Ma, P., DiJohn, D., Tzipori, S. and Tacket, C. O. (1990). Treatment with bovine hyperimmune colostrum of cryptosporidial diarrhea in AIDS patients. *AIDS* **4**, 581–584.

Pantenburg, B., Cabada, M. M. and White, A. C. (2009). Treatment of cryptosporidiosis. *Expert Reviews of Anti Infective Therapy* **7**, 385–391.

Rossignol, J. F. (2009). Thiazolides: a new class of antiviral drugs. *Expert Opinion in Drug Metabolism and Toxicology* **5**, 667–674.

Sinkala, E., Katubulushi, M., Sianongo, S., Obwaller, A. and Kelly, P. (2011). Extreme metabolic disturbances contribute to high mortality in Zambian patients with HIV-related cryptosporidiosis treated with miltefosine. *Annals of Tropical Medicine and Parasitology*, in press.

Siwila, J., Phiri, I. G. K., Vercruysse, J., Goma, F., Gabriel, S., Claerebout, E. and Geurden, T. (2007). Asymptomatic cryptosporidiosis in Zambian dairy farm workers and their household members. *Transactions of the Royal Society of Tropical Medicine* **101**, 733–734.