Medical Sciences (1999), 1, 59-62

Cryptosporidiosis in Children from the Sultanate of Oman

J. J. Windsor[1], S. El-Shafie[2], S. Welch[1] and F. Al-Toqui[1]

[1]Department of Microbiology and Immunology, College of Medicine, Sultan Qaboos University, P.O.Box 35, Al-Khod 123, Muscat, Sultanate of Oman. [2]Department of Medicine Laboratory, Hamad Medical Corporation, P.O. Box 3050, Dohar, Qatar.

البوغ الخفي في سلطنة عُمان

ج . ج ويندزو ، س . شمس الدين الشافي ، وفضيلة سلطان الطوقي

خلاصة : لقد تم خلال عام فحص ٨٠٧ عينة أخذت من أطفال تراوحت أعمارهم بين ٥ أيام إلى ١٢ سنة أُحضروا لإجراء الفحص الميكروبيولوجي الروتيني وللبحث عن حويصلات (أكياس) طفيل البوغ الخفي (*Cryptosporidium*) . لقد فحصت العينات بعد صبغها باليورامين الفينولي (Phenol-auramine) . كما تم قياس الحويصلات بعد التأكد منها بطريقة زيل نيلسن (Ziehl Neelsen) . لقد وجدت حويصلات البوغ الخفي في ١٦ عينة نسبة (١,٩٪) محتلة المرتبة الثالثة بين الطفيليات المعوية الجياردیا (٦٪) وانتميبا النسيج (٢,٣٪) ، وتجدر الإشارة إلى أن اثنين فقط من المصابين بالبوغ الخفي يعانون من النقص المناعي ، كما أنه لم يتم تشخيص أي إصابة بالبوغ الخفي خلال أشهر الصيف الحارة (مايو ويونيو) .

ABSTRACT: Over a one year period, 807 faecal samples from children (0-12 years) were submitted for routine microbiological examination and screening for *Cryptosporidium* oocysts. A Phenol-auramine stain was used and putative oocysts were measured after confirmatory staining with the modified Ziehl Neelsen method. *Cryptosporidium* were identified in 16 (1.9%) of the samples, and was the third most common enteropathogen after *Giardia lamblia* (6.0%) and *Entamoeba histolytica/dispar* (2.3%). With the exception of two patients with underlying immunodeficiency disorders, all were immunocompetent. No cases of *Cryptosporidium* were diagnosed during the hottest months of May and June.

Cryptosporidium is recognised as an important aetiological agent associated with diarrhoeal diseases of humans and animals. It was first described in humans in 1976 (Meisel et al., 1976; Nime et al., 1976). Up until 1982 few cases had been reported, most of which were in patients with underlying immu-nodeficiency disorders. The number of cryptosporidiosis cases rose dramatically with the spread of HIV, the causative agent of Acquired Immunodeficiency Syndrome (AIDS). In 1985, 3.6% of AIDS cases reported to the Centers for Disease Control in the United States were positive for *Cryptosporidium* (Navin, 1985). Since 1980, numerous studies have been performed on immunocompetent patients in Europe (Casemore and Jackson, 1983; Hunt et al., 1984; Baxby and Hart, 1986; Corbett-Feeney, 1987), North America (Current et al., 1983; Ratnam et al., 1985; Wolson et al., 1985), Africa (Bogaerts et al., 1984; Hojlyng et al., 1986; Smith and Van den Ende, 1986; Molbak et al., 1997), Asia (Matran et al., 1985; Shahid et al., 1985), and Australia (Biggs et al., 1987). Many of these studies have shown children to be more commonly infected than adults (Navin, 1985). Some authors have reported that children under two years of age are more susceptible to infection (Baxby and Hart, 1986). *Cryptosporidium* has also been associated with traveller's diarrhoea (Sterling et al., 1986), outbreaks in day-care centres (Alpert et al., 1984), and large outbreaks associated with contamination of water supplies (Hayes et al., 1989; and MacKenzie et al., 1995). Cryptosporidiosis was initially thought to be primarily a zoonosis, but it has been demonstrated that person-to-person transmission is common (Current and Garcia, 1991).

Although a few reports have been published on the incidence of cryptosporidiosis in the Middle East (Khan et al., 1998; Daoud et al., 1990; Bolbol, 1992), no definitive study has been reported from the Sultanate of Oman. The purpose of the present study was to determine the occurrence of *Cryptosporidium* among children in the Sultanate of Oman.

Materials and Methods

During a one year period starting on 1st January 1994, all faecal samples collected from children (0-12 years) presenting to Sultan Qaboos University Hospital (SQUH) were examined for *Cryptosporidium* oocysts.

Air-dried faecal smears were fixed in methanol and stained by the Phenol-auramine method (Fleck and Moody, 1988). Smears were scanned for oocysts by

fluorescent microscopy at 200X and 400X. Samples showing yellow fluorescence with a pale halo were further subjected to the modified Ziehl-Neelsen staining method (Casemore et al., 1984). Oocysts were then measured using an eyepiece graticule (Olympus Optical Co Ltd, Tokyo, Japan). It is essential to measure putative oocysts to distinguish them from *Cyclospora*, which is associated with traveller's diarrhoea (Editorial, 1994). *Cryptosporidium* oocysts measure 4-5 μm whereas *Cyclospora* oocysts are much larger (8-9 μm). The staining characteristics are similar, therefore failure to measure oocysts could result in misdiagnosis. A positive control was used for each batch of smears. All specimens were examined for the presence of *Salmonella* spp, *Shigella* spp and *Campylobacter* spp using standard bacteriological techniques. In addition faecal samples from children under 3 years were examined for enteropathogenic *Escherichia coli*. Saline and iodine wet mounts were examined microscopically for the presence of parasitic cysts and ova.

Results and Discussion

A total of 807 faecal samples, from 768 different patients, were screened over the one year period. Cryptosporidium oocysts were found in 17 stools (2.1%) from 16 patients (1.9%). The children ranged in age from one month to 12 years. Twelve (75%) of the infected patients were less than two years old. Of the 16 patients, 9 were male and 7 were female. In addition to diarrhoea, three patients presented with fever and one with vomiting. Two of these patients were co-infected with a bacterial pathogen, namely *Campylobacter* spp, and *Shigella flexneri* type 6. Two patients were immunocompromised, one with AIDS, the other with hyper IgE syndrome. Most cases presented in the cooler months of December and January, and no cases at all were recorded during the hottest months of May and June (Table 1).

Giardia lamblia was found in 6.0% of the faecal samples examined. Other enteropathogens found are shown in Table 2.

Our results show that cryptosporidiosis is an important cause of gastroenteritis in Oman, and is a commoner pathogen than *Salmonella* spp (1.7%), *Shigella* spp (1.8%), and *Campylobacter* spp (1.1%). It was second in frequency only to *Giardia lamblia* (6.0%) and *Entamoeba histolytica/dispar* (2.3%). Our incidence of 1.9% was slightly higher than that previously reported in Saudi Arabia (1.0 and 1.1%) (Khan et al., 1988; Bolbol, 1992), and Kuwait (1.6%) (Daoud et al., 1990). Some studies in the less developed countries of Africa and Asia have reported an incidence as high as 20% (Current and Garcia, 1991).

Seventy-five percent of the patients in the present study were less than two years of age. Baxby and Hart (1986) in the UK have reported similar findings. Although breast-feeding is encouraged in Oman, many mothers alternate between breast and bottle feeding. This exposure to food and water may be a contributing factor for the high incidence found in this age group. Unfortunately, however,. it was not possible to examine the water

TABLE 1

Monthly cases of cryptosporidiosis

MONTH	NUMBER OF SAMPLES	POSITIVE SAMPLES	% POSITIVE SAMPLES
January	60	4	6.6
February	66	2	3.0
March	47	2	4.2
April	72	2	2.7
May	42	0	0
June	53	0	0
July	74	1	1.3
August	61	0	0
September	104	1	0.9
October	82	0	0
November	79	1	1.2
December	67	3	4.4

TABLE 2

Enteropathogens Recovered.

ORGANISM	NUMBER	PERCENT
Giardia lamblia	49	6.0
Entamoeba histolytica/dispar	19	2.3
Cryptosporidium spp	16	1.9
Salmonella spp	14	1.7
Shigella flexneri	8	0.9
Shigella sonnei	5	0.6
Shigella boydii	2	0.2
Enteropathogenic *Escherichia coli*	12	1.4
Campylobacter spp	9	1.1

supplies from the various regions of the country represented in this study.

No cases of cryptosporidiosis were found during the hottest months of May and June. This absence of cases during the summer months has also been reported in Kuwait (Daoud et al., 1990). Indeed, seasonal variations in the incidence of cryptosporidiosis have been reported in other studies, but the reasons for this phenomenon is still unclear (Baxby and Hart, 1986; Corbett-Feeney, 1987).

The sources of infection in the present study are unknown. It is well established, however, that horizontal transmission among humans is not uncommon and explains the large numbers of cases in urban areas (Casemore, 1986). Cryptosporidia can also be transmitted to humans from domestic animals, such as cattle and sheep (Hayes et al., 1989). It is presently unknown whether the principle livestock animals in Oman, namely goats and camels, are important reservoirs of infection. Cryptosporidia have been described in goats, and an organism described as *C. muris*-like has been reported in a camel (Fayer et al., 1991). We are presently investigating the incidence of cryptosporidiosis in these animal species. There are some reports that indicate that there are antigenic differences between Cryptosporidia found in animals and man (Awad-El-Kariem et al., 1995).

Cryptosporidium oocysts are very resistant in the external environment and can persist in water supplies even after treatment (Hayes et al., 1989). Two large waterborne outbreaks have been reported in the United States, one involving 14,000 people (Hayes et al., 1989), the other involving >400,000 people (MacKenzie et al., 1995). Many residences in Oman are supplied with water from tankers that may be a potential source of infection.

Conclusions

The results of the present study indicate that *Cryptosporidium* is an important pathogen associated with diarrhoea of children in Oman. Indeed, it was second in importance only to *Giardia lamblia* and *Entamoeba histolytica/dispar*. To ascertain the source of infection, future studies will incorporate the investigation of faecal samples of family members of the infected children, livestock and screening of water supplies.

Acknowledgements

The authors wish to thank the laboratory staff in the Department of Microbiology and Immunology for technical assistance.

References

LPERT, G., BELL, L.M., KIRKPATRICK, C.E., BUDNICK, L.D., CAMPOS, J.M., FRIEDMAN, H.M. and PLOTKIN, S.A. (1984). Cryptosporidiosis in a day care centre. *New England Journal of Medicine*, **311**, 860-861.

AWAD-EL-KAREIM, F.A., ROBINSON, H.A., DYSON, D.A., EVANS, D., WRIGHT, S., FOX, M.T. and MCDONALD, V. (1995). Differentiation between human and animal strains of *Cryptosporidium parvum* using isoenzyme typing. *Parasitology*, **110**, 129-132.

BAXBY, D. and HART, C.A. (1986). The incidence of cryptosporidiosis: a two-year prospective survey in a children's hospital. *Journal of Hygiene*, **96**, 107-111.

BIGGS, B.A., MEGNA, M., WICKREMESINGHE, S. and DWYER, B. (1987). Human infection with *Cryptosporidium* spp.: results of a 24-month survey. *Medical Journal of Australia*, **147**, 175-177.

BOGAERTS, J., LEVAGE, P., ROUVROY, D. and VANDEPITTE, J. (1984). *Cryptosporidium* spp, a frequent cause of diarrhoea in Central Africa. *Journal of Clinical Microbiology*, **20**, 874-876.

BOLBOL, A.S. (1992). Cryptosporidiosis in young children suffering from diarrhoea in Riyadh, Saudi Arabia. *Journal of Hygiene, Epidemiology, Microbiology and Immunology*, **4**, 396-400.

CASEMORE, D.P. and JACKSON, B. (1983). Sporadic cryptosporidiosis in children. *Lancet*, **2**, 679.

CASEMORE, D.P., ARMSTRONG, M. and JACKSON, B. (1984). Screening for *Cryptosporidium* in stools. *Lancet*, **1**, 734.

CASEMORE, D.P., ARMSTRONG, M. and SANDS, R.L. (1985). Laboratory diagnosis of cryptosporidiosis. *Journal of Clinical Pathology*, **38**, 1337-1341.

CASEMORE, D.P. (1986). Human cryptosporidiosis. In: *Recent Advances in Infection*, D.S. Reeves and A.D. Geddes (Eds.). Edinburgh, Livingstone (pp 209-236).

CORBETT-FEENEY, G. (1987). *Cryptosporidium* among children with acute diarrhoea in the west of Ireland. *Journal of Infection*, **14**, 79-84.

CURRENT, W.L., REESE, N.C., ERNST, J.V., BAILEY, W.S., HEYMAN, M.B. and WEINSTEIN, W.M. (1983). Human cryptosporidiosis in immunocompetent and immunodeficient persons. *New England Journal of Medicine*, **308**, 1252-1257.

CURRENT. W.L. and GARCIA, L.S. (1991). Cryptosporidiosis. *Clinical Microbiology Reviews*, **4**, 325-358.

DAOUD, A.S., ZAKI, M., PUGH, R.N.H., AL-MUTARI, G., AL-ALI, F. and EL-SALEH, Q. (1990). *Cryptosporidium* gastroenteritis in immunocompetent children from Kuwait. *Tropical Geographical Medicine*, **42**, 113-118.

EDITORIAL (1994). *Cyclospora*: another "new" pathogen. *Journal Medical of Microbiology*, **41**, 217-219.

FAYER, R., LINDSAY, P., ANDERSON, B.C. and BUSH, M. (1991). Chronic cryptosporidiosis in a Bactrian camel (*Camelus bactrianus*). *Journal of Zoology and Wildlife Medicine*, **22**, 228-232.

FLECK, S.L. and MOODY, A.H. (1988). *Diagnostic techniques in Medical Parasitology*. London: Butterworths.

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

HOJLYNG, N., MOLBAK, K. and JEPSEN, S. (1986). *Cryptosporidium* spp, a frequent cause of diarrhoea in Liberian children. *Journal of Clinical Microbiology*, **23**, 1109-1113.

HUNT, D.A., SHANNON, R., PALMER, S.R. and JEPHCOTT, A.E. (1984). Cryptosporidiosis in an urban community. *British Medical Journal*, **289**, 814-816.

KHAN, Z.H., NAMNYAK, S.S., AL JAMA, A.A. and MADAN, I. (1988). Prevalence of cryptosporidiosis in Damman and Alkhobar, Saudi Arabia. *Annals of Tropical Paediatrics*, **8**, 170-

172.

MACKENZIE, W.R., SCHELL, W.L., BLAIR, K.A., ADDISS, D.G., PETERSON, D.E., HOXIE, N.J., KAZMIERCZAK, J.J. and DAVIS, J.P. (1995). Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. *Clinical Infectious Diseases*, **21**, 57-62.

MATA, L.B., BOLANOS, H., PIZARRO, D. and VIVES, M. (1984). Cryptosporidiosis in children from highland Costa Rica rural and urban areas. *American Journal of Tropical Medicine and Hygiene*, **33**, 24-29.

MATRAN, M.M., VENKATESAN, S., GEORGE, R., MATHEW, M. and MATHAN, V.I. (1985). *Cryptosporidium* and diarrhoea in Southern Indian children. *Lancet*, **2**, 1172-1175.

MEISEL, J.L., PERERA, D.R., MELIGRO, C. and RUBIN, C.E. (1976). Overwhelming watery diarrhoea associated with *Cryptosporidium* in an immunosuppressed patient. *Gastroenterology*, **70**, 1156-1160.

MOLBAK, K., ANDERSEN, M., AABY, P., HOJLYNG, 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.

NAVIN, T.R. (1985). Cryptosporidiosis in humans: review of recent epidemiologic studies. *European Journal of Epidemiology*, **1**, 77-83.

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

RATNAM, S., PADDOCK, J., MCDONALD, E., WHITTY, D. JONG, M. and Cooper, R. (1985). Occurrence of *Cryptosporidium oocysts* in faecal samples submitted for routine microbiological examination. *Journal of Clinical Microbiology*, **22**, 402-404.

SHAHID, N.S., RAHAMAN, A.S.M.H., ANDERSON, B.C., MATA, L.J. and SANYAL, S.C. (1985). Cryptosporidiosis in Bangladesh. *British Medical Journal*, **290**, 114-115.

SMITH, G., and VAN DEN ENDE, J. (1986). Cryptosporidiosis among black children in hospital in South Africa. *Journal of Infection*, **13**, 25-30.

STERLING, C.R., SEEGAR, K. and SINCLAIR, N.A. (1986). *Cryptosporidium* as a causative agent of traveller's diarrhoea. *Journal of Infectious Diseases*, **153**, 380-381.

WEBER, R., BRYAN, R.T. and JURANEK, D.D. (1992). Improved stool concentration procedure for detection of *Cryptosporidium* oocysts in faecal specimens. *Journal of Clinical Microbiology*, **30**, 2869-2873.

WOLSON, J.S., RICHTER, J.M., WALDRON, M.A., WEBER, D.J. MCCARTHY, D.M. and HOPKINS, C.C. (1985). Cryptosporidiosis in immunocompetent patients. *New England of Journal Medicine*, **312**, 1278-1282.