Epidemiol. Infect. (2001), **126**, 301–307. Printed in the United Kingdom

Paired city *Cryptosporidium* serosurvey in the southwest USA

F. J. FROST[1]*, T. MULLER[1], G. F. CRAUN[2], R. L. CALDERON AND P. A. ROEFER[4]

[1] *The Lovelace Clinic Foundation, 2425 Ridgecrest Drive S.E. Albuquerque, 87108 USA*
[2] *101 West Frederick Street, The Professional Building, Staunton, VA, 24401*
[3] *National Health and Environmental Effects Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27111*
[4] *Southern Nevada Water Authority, 243 Lakeshore Road, Boulder City, NV 89005*

(*Accepted 24 October 2000*)

SUMMARY

In 1996, serological responses to two *Cryptosporidium* antigens were determined for 200 Las Vegas (LV), Nevada, and 200 Albuquerque, New Mexico, blood donors to evaluate associations between endemic infections, water exposures, and other risk factors. LV uses chlorinated filtered drinking water from Lake Mead while Albuquerque uses chlorinated ground water. The intensity of serological response to both markers was higher for older donors ($P < 0{\cdot}05$), donors who washed food with bottled water ($P < 0{\cdot}05$) and donors from LV ($P < 0{\cdot}05$). A decreased serological response was not associated with bottled water consumption, nor was an increased response associated with self-reported cryptosporidiosis-like illness or residence in LV at the time of a cryptosporidiosis outbreak 2 years earlier. Although these findings suggest the serological response may be associated with type of tap water and certain foods, additional research is needed to clarify the role of both food and drinking water in endemic *Cryptosporidium* infection.

INTRODUCTION

In 1992, cryptosporidiosis was made a reportable disease in Nevada and three cases were reported that year. In 1993, 23 case reports were received with 18 of these cases known to be HIV infected. The identification of 78 laboratory confirmed cryptosporidiosis cases in the first 4 months of 1994 alerted public health officials to the occurrence of an outbreak in Clark County which includes Las Vegas (LV), Nevada [1]. The number of case reports began to increase in January, 1994, peaking in March. Between January and April, 1994, 78 cases of cryptosporidiosis were reported from Clark County [1] and an additional 49 cases were reported from May to December. As with the prior year, most cases diagnosed during the first quarter of 1994 were immunosuppressed persons or young children. A case-control study of 36 HIV-infected adults diagnosed with cryptosporidiosis by stool examination during the first 4 months of 1994 and HIV-infected persons not diagnosed with cryptosporidiosis (108 controls) was conducted in May–June 1994, approximately 100 days following the onset of illnesses. Exclusive use of bottled water at the time of the case's illness was reported significantly more often by controls (HIV-infected persons with $CD4^+$ counts below 100) than cases (odds ratio = 13·52; 95% CI = [1·78, 102·92]) [1]. No association was found between cryptosporidiosis and reported food, travel, recreation, or animal exposures. An external review of the outbreak investigations questioned whether recall bias may have influenced reporting of bottled water use [2].

* Author for correspondence.

The designation of this outbreak as waterborne suggested that cities using relatively uncontaminated surface-derived source water and applying direct filtration water treatment may still be at risk for waterborne cryptosporidiosis outbreaks, even though all water quality standards are met and operational/monitoring measurements detected no water quality problems. Source water testing in LV during previous months had not detected *Cryptosporidium* oocysts [3]. An investigation of the Alfred Merit Smith Water Treatment Plant (AMSWTP) failed to find water treatment deficiencies, and water quality monitoring records indicated no temporal changes in the finished water quality during 1994. Based on continuous turbidity measurements, the maximum raw water turbidity was 0·26 NTU and the maximum treated water turbidity was 0·17 NTU for the period 12/93–4/94. These levels are well below United States drinking water standards, requiring that 95% of monthly measurements of treated drinking water be below 0·5 NTU [1]. A maximum of 7 coliforms per 100 ml were found in raw water, while no coliforms were detected in the finished water. Many water utilities use more heavily contaminated surface water sources and few maintain the treatment performance of the AMSWTP.

Since the year of the outbreak the number of reported cryptosporidiosis cases in Clark County has declined dramatically (22 cases in 1995, 17 cases in 1996, 9 cases in 1997, 4 cases in 1998 and 6 in 1999). However, raw and finished drinking water quality indices and water treatment processes and operation have not changed significantly since before the outbreak. We conducted a paired-city serological study of *Cryptosporidium* infections and risk factors for infection. Evidence of prior *Cryptosporidium* infections was obtained from serological responses to two *Cryptosporidium* antigens among blood donors from Las Vegas, NV and Albuquerque, NM. These two cities are similar large urban centres in the arid United States Southwest. However, Albuquerque obtains almost 100% of its drinking water from a deep underground source, whereas 85% of Clark County residents use surface water from Lake Mead, which is part of the Colorado River system. Our hypothesis was that if drinking water is a continuing route of *Cryptosporidium* transmission in LV and other risk factors for infection are comparable for the two populations, then serological responses should be more intense and/or more frequent among LV residents compared to Albuquerque residents.

METHODS

Study population

Four hundred volunteer blood donors age 19–69 years in Bernalillo County, New Mexico (200) and Clark County, Nevada (200) were recruited to participate in the study. Participants signed an informed consent and completed a 2-page questionnaire that asked about age, sex, race, city of residence, duration of residence in that city, source of drinking water, and whether their home had a filter for drinking water. Participants were asked whether they regularly drink bottled water and, if so, what percentage of all drinking is from bottled water. They were also asked whether they use bottled water for making ice and for washing food. They were asked whether they had ever been diagnosed with cryptosporidiosis or if anyone in their home had been diagnosed with cryptosporidiosis. Regarding the prior 12 months, participants were asked whether they had children under age 5 in the household, whether the children attended day care, whether they had young or adult household pets, whether they had exposure to livestock or zoo animals, swam in a public pool, lake or river or traveled to a foreign country. They were asked whether, during the past 2 months, they had experienced diarrhoea lasting for 4 or more days. Reported bottled water use was coded as some (< 70% of drinking water), most (70–99%) and all (100%). Following the blood draw, sera were separated, frozen and stored at the facility. The batched sera were then shipped to the Lovelace Institutes in Albuquerque, New Mexico. IgG serological response was determined using a Western blot assay.

Western blot procedures

Sera were collected and analysed by immunoblot to measure IgG serological response to the 15/17- and 27-kDa antigen groups. This method, which uses the miniblot format, has been described elsewhere [4, 5]. The intensity of the serological responses to the 15/17- and 27 kDa antigen groups on the immunoblots are digitally analysed by an IS-2000 Digital Imaging System (Alpha Innotech). Serological response to the two antigen groups was based on the measured area under the curve of response intensity for each lane at the expected location of the response for the antigen group.

Table 1. *Demographic factors that differ among study participants in Las Vegas and Albuquerque*

	Las Vegas ($n = 200$) (%)	Albuquerque ($n = 200$) (%)	*P*-value
Use bottled water for drinking	50	20	$< 0{\cdot}0001$
Use bottled water for making ice	19	4	$< 0{\cdot}001$
Use bottled water for washing food	5	1	$< 0{\cdot}05$
Had livestock or zoo animal exposure in the past 12 months	5	19	$< 0{\cdot}05$
Work in Las Vegas or Albuquerque	94	83	$< 0{\cdot}01$

Table 2. *Tobit analysis. Factors in addition to age associated with increased risk of serological response*

Factors in the model	15/17 kDa *P*-value	27 kDa *P*-value
Wash food with bottled water	0·005	0·04
Residence in Las Vegas	0·0004	0·05
Resident of Las Vegas for < 3 years	0·70	0·67
Drank water from stream, lake	0·09	0·50
Swam in lake or pool	0·55	0·29
Young pets in household	0·04	0·28
Bottled water or home filter for:		
1–69% of drinking water	0·88	0·94
70–99% of drinking water	0·45	0·82
100% of drinking water	0·52	0·77
Foreign travel	0·82	0·56
Children < 5 in household	0·18	0·19
White race	0·30	0·50
Female sex	0·76	0·61
Age *vs.* less than age 30		
Age 30–39	0·08	0·01
Age 40–49	0·02	0·01
Age 50–59	0·002	0·01
Age 60+	0·002	0·03

The IgG results for each specimen were standardized by taking the ratio of the response intensity for the unknown sample to the response intensity for a positive control serum contained on each blot. The IgG positive control sera were obtained from individuals with a strong serological response to both antigens, approximating the intensity of responses observed from several individuals with laboratory confirmed cryptosporidiosis. The same positive control sera were used on all blots.

Analysis

Multivariate analysis of the observed serological response intensities was conducted using a Tobit model [6]. The Tobit model assumes an underlying normal distribution of the responses about the mean intensity, but allows a point mass at zero. Since the distribution of serological responses was skewed, with a right tail, the responses were transformed using a square root to better approximate a normal distribution. The Tobit model was fit to both square root transformed and untransformed data using SAS proc lifereg.

Case reports of diagnosed cryptosporidiosis were obtained from the Nevada Health Division and the New Mexico Health Department. Incidence rates were calculating using yearly estimated population counts from the US Bureau of the Census.

RESULTS

Sera samples were collected during 9/96–2/97. Both donor populations were similar with respect to age, sex, race, marital status, residence in the city versus outside the city, duration of residence in the area, number of children and/or pets in the household, and histories of travel, swimming, untreated water consumption, prolonged diarrhoea or contact with someone suffering from diarrhoea. Most Bernalillo County donors resided in Albuquerque (83%) and most Clark County donors resided in LV (94%). For this reason, these groups will be referred to as either Albuquerque or LV donors. Albuquerque donors were more likely to work in a city other than their city of residence ($P < 0{\cdot}01$) and more likely to have contact with cattle ($P < 0{\cdot}05$) than Las Vegas donors. Las Vegas donors were more likely to have a home water treatment system or use bottled water for drinking ($P < 0{\cdot}0001$), making ice ($P < 0{\cdot}001$) and washing food ($P < 0{\cdot}05$) (Table 1).

A detectable response to the 15/17 kDa antigen group was observed in 49% of LV donors and in 36%

https://doi.org/10.1017/S0950268801005337 Published online by Cambridge University Press

Table 3. *Rates of reported cryptosporidiosis*: *Bernalillo County*, *New Mexico* vs *Clark County*, *Nevada*

	Cryptosporidiosis incidence rates per 100000 population	
Year	Bernalillo County Albuquerque (*n*)	Clark County Las Vegas (*n*)
1993	3·8 (19)	2·7 (23)
1994	5·9 (30)	14·7 (127)
1995	3·5 (18)	2·3 (22)
1996	3·1 (16)	1·7 (17)
1997	7·2 (38)	0·9 (9)
1998	3·0 (16)	0·4 (4)
1999	4·0 (21)	0·5 (6)

of Albuquerque donors ($P < 0{\cdot}01$, relative prevalence (RP) = 1·36, 95% C.I. [1·08, 1·73]). Defining a positive response as greater than 10%, 20%, 35% or 50% all gave statistically significant differences between LV and Albuquerque (10%, RP = 1·31 [1·02, 1·67]; 20%, RP = 1·59 [1·19, 2·12]; 35%, RP = 2·17 [1·49, 3·16]; 50%, RP = 2·17 [1·35, 3·50]). For the 27 kDa antigen, defining a positive response as detectable, 10% or 20% of the positive control did not show statistically significant differences between LV and Albuquerque (detectable, RP = 1·01 [0·85, 1·20]; 10%, RP = 1·01 [0·85, 1·20]; 20%, RP = 1·17 [0·93, 1·49]). However, defining a positive as 35% or 50% of the positive control did show statistically significant differences in serological responses between LV and Albuquerque (35%, RP = 1·62 [1·18, 2·23]; 50%, RP = 1·88 [1·25, 2·81]).

Eleven donors reported using bottled water for washing food and nine of them had a detectable response to both the 15/17- and 27-kDa markers. Defining a positive response for the 15/17 kDa marker as a detectable response, 10%, 20%, 35%, or 50% of the positive control all gave a statistically significant higher rates of positivity for people who washed food with bottled water (RP = 1·05–1·08 [1·01, 1·09–1·17]). A weaker relationship was observed for response to the 27 kDa marker for each definition (RP = 1·02–1·04, [0·99, 1·06–1·10]).

Multivariate Tobit analyses were conducted separately for the 15/17 kDa and 27 kDa markers to determine if differences in other risk factors may have affected the comparisons of serological responses between LV and Albuquerque. The Tobit analysis was used because serological responses are left censored at zero. Over half of serological responses to the 15/17 kDa antigen were zero (51% for LV, 64% for Albuquerque). The multivariate analyses also indicated that Las Vegas donors had a more intense response to both serological markers than did Albuquerque donors ($P < 0{\cdot}05$) (Table 2). Increased age and washing food with bottled water were associated with a more intense serological response to both markers (Tables 2, 3) ($P < 0{\cdot}05$). Having a young child in the household was associated with a more intense response to both markers but the difference was not statistically significant ($P > 0{\cdot}19$). Donors who moved to Las Vegas after the 1994 outbreak, did not have a less intense serological response to either marker ($P > 0{\cdot}60$), suggesting that serological responses observed in LV donors who were residents during the outbreak were not higher than those who moved to LV after the outbreak. Use of bottled water for some, most or all of drinking water or having a home filter for drinking water was not associated with a lower serological response for donors from either city ($P > 0{\cdot}45$).

During 1993–9, the incidence of laboratory-diagnosed and reported cryptosporidiosis was higher in Bernalillo County, New Mexico, than in Clark County, Nevada, for each year except for the year of the outbreak (Fig. 1). As the incidence of cryptosporidiosis in Clark County declined in recent years, the differences in incidence between Clark and Bernalillo counties increased. Cryptosporidiosis is a reportable disease in both Nevada and New Mexico and residents of both Bernalillo and Clark counties have comparable access to health care. Both the Clark County and the New Mexico Department of Health routinely contact all cases of cryptosporidiosis reported by laboratories. Most laboratory testing for *Cryptosporidium* appears to occur in two laboratories in Clark County whereas three laboratories are

https://doi.org/10.1017/S0950268801005337 Published online by Cambridge University Press

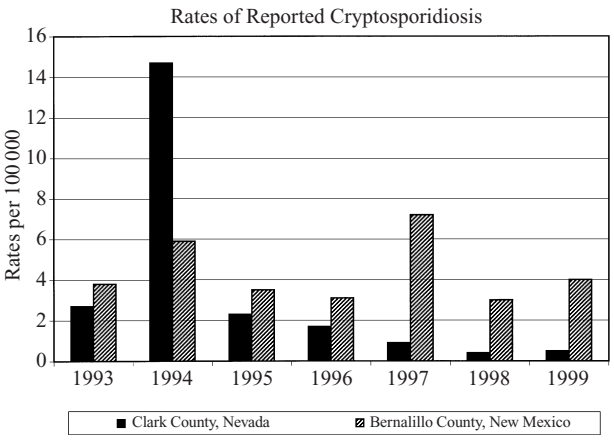

Fig. 1. Cryptosporidiosis rates by year.

responsible for *Cryptosporidium* testing in Bernalillo County. Rates of *Cryptosporidium* testing per 1000 population are not available for either county.

DISCUSSION

This finding of a more frequent serological response to one antigen group and more intense responses to both groups among blood donors from a surface versus a ground water city is consistent with a prior study conducted in the US Midwest [7]. These two studies provide suggestive evidence that an elevated level of endemic waterborne *Cryptosporidium* infections occurs among users of surface-derived drinking water. Serological responses to the 15/17 kDa antigen group (greater than or equal to 10% of the positive control) were detected among 35% of Albuquerque and 46% LV blood donors, compared to 53% for blood donors from the Midwest city that uses filtered and chlorinated water from a major river system that has multiple sources of both human and animal sewage upstream and 39% of donors from a Midwest city that uses a high quality ground water source [7]. The occurrence of serological responses to the 27 kDa antigen group for both Albuquerque and Las Vegas and for the Midwest surface water city were comparable (52% for Albuquerque, 55% for LV, 49% for the Midwest surface water city) and higher than found in the Midwest ground water city (36%).

Alternatively, the higher serological responses observed in Las Vegas versus Albuquerque blood donors (and for the 2 Midwest cities) may have been due to factors other than the drinking water. The failure to observe a lower serological response among people who regularly drank bottled water in Las Vegas and Albuquerque raises questions about a causal relationship between the drinking water source and an increased risk of infection. Although foods and food sources have not been commonly linked to cryptosporidiosis outbreaks, food contamination should be considered as an alternative source of infection. Because of the diversity of sources of fresh fruits and vegetables, establishing an epidemiological relationship between a food source and infections or outbreaks can be particularly challenging. It is also possible that risk factors not considered by this study may have caused differences between cities in the prevalence of serological responses to infection.

Because of the occurrence of the 1994 LV cryptosporidiosis outbreak, one might expect that LV medical practitioners and the general public, including the LV blood donors participating in this study, would more likely suspect and report symptoms consistent with cryptosporidiosis than residents of other cities, such as Albuquerque. If so, this would increase the likelihood of detecting ongoing occurrences of cryptosporidiosis in LV and observing a relationship between symptoms and serological response to *Cryptosporidium* markers among LV blood donors. However, the incidence of diagnosed cryptosporidiosis each year since the outbreak has been lower in LV than in Albuquerque. Alternatively, it is possible that *Cryptosporidium* testing occurs more commonly in Albuquerque than in LV or Albuquerque physicians may better identify high risk patients for testing or Albuquerque laboratories may be more proficient in detecting the infection. These and other reporting factors could account for the higher rates of cryptosporidiosis reported in Albuquerque.

Among donors from either city there was no relationship between the occurrence of symptoms consistent with cryptosporidiosis during the past 6 months and serological response to either marker. It is possible that mild illness may have been associated with strong serological responses but that these symptoms would not have been recalled at the time of the blood draw which occurred some weeks later. Alternatively, it is possible that the more intense serological responses to the *Cryptosporidium* antigens among LV residents, observed in this study, indicate an increased level of protection from clinically significant cryptosporidiosis among LV residents due to higher levels of endemic infections [8–11]. In a study of experimentally infected individuals, seroconversion was observed in 10 of 11 symptomatic

https://doi.org/10.1017/S0950268801005337 Published online by Cambridge University Press

cases. The response peaked 1 month following infection and declined thereafter, however, a small increased response was observed up to 1 year later [8]. For the entire cohort, the presence of prior serological response was associated with a lower likelihood of reported illness following infection [8].

The high rate of home filter or bottled water use among LV survey donors may have resulted from concerns about the safety of the LV water supply, because of the 1994 outbreak. However, use of a home water filter or bottled water, even for 100% of drinking water, did not reduce the intensity of serological responses to the 2 *Cryptosporidium* markers. The failure of bottled water use to reduce the serological responses to *Cryptosporidium* is unexpected, if drinking water is assumed to be a risk factor for endemic transmission. The 1994 outbreak investigation found that the exclusive use of bottled water was protective for cryptosporidiosis among HIV-AIDS cases. It is possible that the higher levels of endemic *Cryptosporidium* infections observed in this study were not due to drinking water.

Alternatively, it is also possible that exposure to tap water may be more common than reported by bottled water users. Davis et al. [12] found that even motivated bottled water users tend to under-report tap water consumption unless special efforts were made to determine all of their sources of drinking water. Comparisons with outbreak data are further complicated because during many suspected waterborne outbreaks, including the LV outbreak, the public and affected persons are aware that drinking water is a likely source of the illnesses. Therefore, people with cryptosporidiosis may be more motivated to recall tap water exposure than controls. In the case of LV, 1 month before the initiation of data collection on bottled water use in the outbreak investigation, HIV-AIDS cases were told by their physicians and the health department to avoid tap water exposure [13]. Since HIV-AIDS cases were selected as the controls for the outbreak case-control study, such advice may have influenced the reporting of bottled water use. It is possible that the observed protective effect of bottled water was an artifact associated with biased recall of exposures between cases and controls or changes in the use of bottled water by controls following advice from their physician to avoid tap water. Had serological data been available for cases and controls at the time of the outbreak, questions about whether recall bias affected the findings of the outbreak investigations may have been resolved.

The findings of a relationship between exposure to children and an increased risk of *Cryptosporidium* infection has been previously demonstrated [14]. The relationship between bottled water use for washing fruit and vegetables and an increased risk of infection merit further inquiry. It is possible that people who use bottled water to wash fruit and vegetables use less water than people who use tap water to wash food. If this is the case and if contaminated fresh fruit and vegetables are a vehicle of *Cryptosporidium* transmission, then how uncooked fruits and vegetable are washed may affect the risk of infection.

Additional studies of this type are needed to ascertain whether elevated risks of *Cryptosporidium* infection are associated with waterborne exposures from surface water sources. The role of source water protection is also an important consideration, even for filtered surface water systems. In particular, are cities using relatively uncontaminated surface water sources, such as LV, at equal risk of endemic waterborne infections as cities using more heavily contaminated surface sources?

Several questions asked of survey participants may have been subject to recall bias or inaccurate reporting. In particular, a single question on bottled water use may not accurately reflect their true consumption of bottled water. Estimates of the percentage of drinking water from bottled sources may also be inaccurate. Reports of diarrhoea over the past 2 months may be incomplete, since it is unknown what the period of accurate recall is for episodes of diarrhoea. Incomplete reporting of swimming and consumption of untreated water from lakes or streams may also occur when reported for a 12-month period.

If endemic *Cryptosporidium* infections occurred at high levels in LV prior to the 1994 outbreak, then this may have reduced the susceptible population for the outbreak. If so, this may have played a role in limiting illness to young children who likely had an insufficient number of prior infections to protect them from cryptosporidiosis and to HIV-AIDS cases who are likely to be at extreme risk of adverse outcomes from infection. The decline in cryptosporidiosis cases in LV in recent years may have resulted from improved treatments for HIV-AIDS. Additional research is needed to improve our understanding of the sources and effects of endemic waterborne *Cryptosporidium* infections and whether high levels of endemic infection influences the characteristics of outbreaks and diagnosed cases in a community.

ACKNOWLEDGEMENTS

This work was supported by the US Environmental Protection Agency through CR-824167-02-0 to the Lovelace Institutes and by the Southern Nevada Water Authority. The views expressed in this article are those of the individual authors and do not necessarily reflect the views and policies of the US Environmental Protection Agency or the Southern Nevada Water Authority. It has been subject to the Agency's peer and administrative review, and it has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This project was made possible by the cooperation of the United Blood Services both in Albuquerque, NM and Las Vegas, NV.

REFERENCES

1. Goldstein ST, Juranek DD, Ravenholt O, et al. Cryptosporidiosis: An outbreak associated with drinking water despite state-of-the-art water treatment. Annals Int Med 1996; **124**: 459–68.
2. Las Vegas Cryptosporidiosis Review Panel Report, 1995. California-Nevada Section AWWA, Southern Nevada Water Authority, Las Vegas Valley Water District, and AWWA Research Foundation.
3. Roefer P, Monscvitz JT, Rexing DJ. The Las Vegas cryptosporidiosis outbreak. JAWWA 1996; **88**: 95–106.
4. Frost FJ, de la Cruz AA, Moss DM, Curry M, Calderon RL. Comparisons of ELISA and Western blot assays for detection of *Cryptosporidium* antibody. Epidemiol Infect 1998; **121**: 205–11.
5. Frost FJ, Calderon RL, Muller TM, et al. A two-year follow-up of antibody to *Cryptosporidium* in Jackson County, Oregon following an outbreak of waterborne disease. Epidemiol Infect 1998; **121**: 213–7.
6. Tobin J. Estimation of relationships for limited dependent variables. Econometrica 1958; **26**: 24–6.
7. Frost F. Two-city *Cryptosporidium* study. American Water Works Association Research Foundation. Drinking Water Res 1998; **8**: 2–5.
8. Moss DM, Chappel CL, Okhuysen PC, et al. The antibody response to 27-, 17- and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. J Infect Dis 1998; **178**: 827–33.
9. Frost FJ, Craun GL, Calderon CL, Hubbs SA. So many oocysts, so few outbreaks. J Am Water Works Assoc 1997; **89**: 8–9.
10. Okhuysen PC, Chappell CL, Sterling CR, Jakubowski W, DuPont HL. Susceptibility and serological response of health adults to re-infection with Cryptosporidium parvum. Infect Immun 1998; **66**: 441–3.
11. Frost FJ, Craun GF. Serological response to human Cryptosporidium infection. Infect Immun 1998; **66**: 4008.
12. Davis LJ, Roberts HL, Juranek DD, Framm SR, Soave R. A survey of risk factors for cryptosporidiosis in New York City: drinking water and other exposures. Epidemiol Infect 1998; **54**: 549–52.
13. The Las Vegas Sun, 9 April 1994.
14. Casemore DP. Human cryptosporidiosis. In: Reeves D, Geddes A, eds. Recent advances in infection. No. 3. Edinburgh, Scotland: Churchill Livingstone, 1998: 209–36.