High serological response to *Cryptosporidium*-specific antigens in the Czech Republic and its association with water supply

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ABSTRACT

A survey was conducted in the Czech Republic to determine whether serological responses to the 15/17-kDa and 27-kDa *Cryptosporidium* antigens had changed since the end of the communist era and if these responses were associated with drinking water sources. Sera from 301 blood donors residing in six areas served by various sources of drinking water were analysed by Western Blot (mini-immunoblots) to measure the IgG response. The intensity of response and percentage of persons with a strong response to the 27-kDa, but not the 15/17-kDa, antigen were higher than found 20 years earlier. A strong response to both the 15/17- and 27-kDa-antigens was higher than reported in other countries, and the probability of persons having a strong response was greater in areas with surface water sources than river-bank infiltration. Few cases of cryptosporidiosis were reported in spite of these high responses to *Cryptosporidium* antigens. These responses suggest a chronic low-level exposure from several sources that may be affording protection against symptoms and illness. Although strong serological responses were associated with surface water sources, drinking water is not likely to be the most important exposure for *Cryptosporidium* in the Czech Republic.

Key words | *Cryptosporidium*, serology, waterborne infections

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INTRODUCTION

Assessing exposures of waterborne contaminants such as *Cryptosporidium* can be difficult, especially when their occurrence is highly variable. Monitoring can be expensive, and sample collection, if it is too infrequent, may not be sufficient to estimate waterborne health risks. Modelling may not be sufficiently precise or optimal. This is why biomarkers are increasingly used to assess environmental exposures and risks. Although biomarkers are usually employed for chemical contaminants, a serological response can be a useful biomarker for microbial contaminants, especially *Cryptosporidium*.

Cryptosporidium infection elicits a serological response in most infected humans, and surveys have estimated the prevalence in various populations, intentionally (Chappell *et al.* 1998, 2006) or unintentionally, exposed to oocysts (Moss *et al.* 1998; Frost *et al.* 2000b). Serological studies using mini-immunoblots have focused on IgG serological responses to

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the 15/17-kDa and 27-kDa antigen groups (Isaac-Renton *et al.* 1999; Frost *et al.* 2003). Infection usually elicits a serological response to these antigen groups that peaks within 14 weeks after infection (Muller *et al.* 2001; Priest *et al.* 2001; Chalmers *et al.* 2013). In the absence of continuing exposures, the 15/17-kDa marker declined to baseline levels observed prior to the infection by 42 weeks after infection while the 27-kDa marker remained elevated more than one year (Muller *et al.* 2001). Studies have found that the type of drinking-water source (e.g., surface water vs groundwater) and/or water-treatment procedures are related to the detection and the density of oocysts in treated drinking water (Lechevallier *et al.* 1991; Gallas-Lindemann *et al.* 2013), as well as the serological response (Frost *et al.* 2003; Ramsay *et al.* 2014).

Previously, using serum samples collected in 1985, we investigated the seroprevalence of the 15/17-kDa and 27-kDa antigens in persons residing in four areas of the Czech Republic with different sources of drinking water or water treatments (Kozisek *et al.* 2008). Among persons surveyed in the area with river-bank infiltration water, 33% had a strong serological response (≥20% of the positive control) to the 15/17-kDa antigen group, whereas over 72% of persons in the other three areas had a strong response. We suspected that the elevated serological responses were associated with drinking water exposures to *Cryptosporidium* because water sources in these three areas were more vulnerable to faecal contamination than the river-bank infiltration source. Conventionally treated surface water was supplied to persons in two of the areas, and persons in the third area obtained groundwater from wells in a fractured sandstone aquifer.

Major political, social and economic changes have occurred in the Czech Republic since 1985, and these changes may have affected *Cryptosporidium* exposure and seroprevalence. The quality of surface waters has improved substantially because of new wastewater treatment facilities, and there have been investments in drinking water treatment technologies. In addition, bottled water consumption has increased. We hypothesized lower *Cryptosporidium* exposure from drinking water and thus, a lower seroprevalence. During December 2004 and January 2005, we conducted a new survey. Serum samples were collected from blood donors from all four of the previously surveyed areas as well as from two additional areas.

MATERIAL AND METHODS

Study sites and type of water supply

The six areas chosen for the survey are cities or groups of cities (Figure 1) with various numbers of inhabitants and

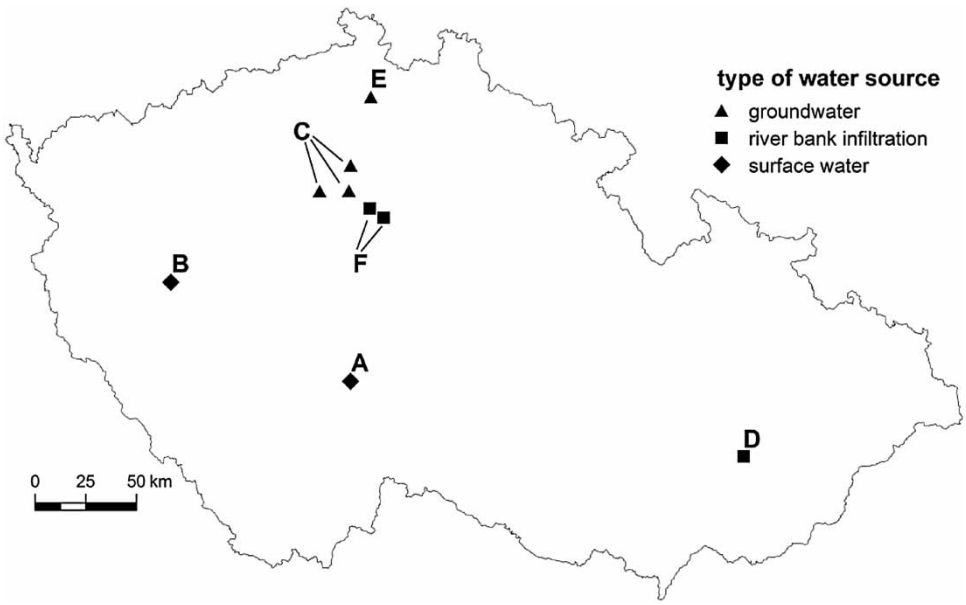

Figure 1 | Map of the Czech Republic with the sites included in the survey.

Table 1 | Population and basic characteristics of water systems of survey areas

Site	Source	Treatment	Disinfection	Population
A	Surface water (reservoir)	Chemical coagulation, sedimentation, sand filtration	Chlorine	33,000
B	Surface water (river)	Chemical coagulation sedimentation, sand filtration, ozone	Chlorine	195,000
C	Groundwater (sandstone aquifer)	None	Chlorine	20,000 (3 cities)
D	River-bank infiltration	None for 50% of flow; rapid sand filtration for 20% of flow; aeration coagulation, sedimentation, rapid sand filtration for 30% of flow; water is then blended at a single facility	Chlorine	26,000
E	Groundwater (deep chalk aquifer)	Aeration and sand filtration for iron removal	Chlorine	40,000
F	River-bank infiltration, mixture of river-bank infiltration and groundwater	None	Chlorine	25,500 (2 cities)

different types of public drinking water systems (Table 1). Sites A–D were included in our previous survey (Kozisek *et al.* 2008). Sites A (Tábor) and B (Plzeň) use surface water sources, but the source of drinking water for site A was changed in 2001. Sites C (Mělník, Neratovice and Kralupy nad Vltavou) and E (Česká Lípa) use groundwater sources and sites D (Kroměříž) and F (Brandýs nad Labem – Stará Boleslav and Čelákovice) use river-bank infiltration. At site F, approximately 40% of the water is from the artificial infiltration of river water after treatment for algae growth control and rapid sand filtration; the remaining 60% is natural infiltration from a nearby river. At site D, all source water is from natural infiltration. More detailed information on study areas, water quality and water treatment is provided in the Supplementary Material, available with the online version of this paper.

Source of the sera

In cooperation with blood banks, we collected a total of 301 serum samples from donors during December 2004 and January 2005. The donors were informed about the study and were asked to complete a short questionnaire to obtain information about age, gender, using water from a public water system and occupation. Free and informed consent of the participants was obtained and the study protocol was approved by the Ethical Committee of the National Institute of Public Health, Czech Republic, before the beginning of the study. Donors were also asked whether or not they commuted to work or school in another city. We used this as a surrogate for exposure to the public water system of their residence; an answer of 'yes' was considered in our analysis as suggestive of less exposure to their public water system than for those who answered 'no'. The survey was designed to collect approximately 50 samples from each of the six sites. The number of samples from each site is noted in Table 2. Two samples were excluded – one due to unsuccessful analytical process and the second, because the donor lived outside site A and used a private well as a source of water. The grouping of samples was comparable according to gender (male to female ratio 1:1) and age (18 to 57 years with the majority 20 to 49 years of age). Although the age distribution of the participants in the six areas of this survey was slightly different from the 1985 survey (Kozisek *et al.* 2008), there was no statistically significant difference in the age distribution for the four areas included in both surveys ($p = 0.286$).

Table 2 | Median *Cryptosporidium* antigen response (% of PC) and percentage of persons with a strong response (2004–2005)

		Median response (95% CI)		Percentage with a strong response (≥20% PC)	
Site	N	15/17-kDa	27-kDa	15/17-kDa	27-kDa
A (SW)	49	59 (36–94)	69 (53–82)	78	92
B (SW)	50	47 (41–61)	84 (63–96)	78	94
C (GW)	50	39 (19–58)	56 (49–73)	62	92
D (BF)	49	20 (9–33)	37 (28–55)	49	73
E (GW)	52	25 (19–50)	59 (48–67)	63	92
F (BF)	49	52 (31–70)	73 (58–82)	73	84
All	299	40 (32–47)	61 (56–68)	67	88

PC, positive control; SW, surface water; GW, groundwater; BF, river-bank infiltration; N, number of samples; 95% CI, 95% confidence interval.

Sera analysis

Sera were analysed by western blot (mini-immunoblots) to measure IgG serological response to the 15/17-kDa and 27-kDa *Cryptosporidium* antigen groups. The analytical method is described in detail elsewhere (Frost *et al.* 2003). The intensity of serological responses is expressed as the percentage of the positive control (PC) for each participant. To assure comparability with our previous survey (Kozisek *et al.* 2008) and other studies (summarized in Frost *et al.* (2003)), we used positive controls of comparable intensity of serological responses. The initial control sera were obtained from the Centers for Disease Control (Atlanta, GA, USA). Subsequent control sera were mixed to obtain serological responses similar to the initial control sera. Quality control procedures included a duplicate positive control and a duplicate randomly selected unknown serum sample for each blot. Serological responses were categorized as non-detectable, detectable with a response of <20% of the positive control (weak response) and ≥20% of the positive control (strong response) (Frost *et al.* 2005b).

Statistical procedures

The distribution of antibody response was found to be skewed in the current study and thus, medians and geometric means were used as measures of central tendency. Medians are supplemented by exact 95% confidence intervals based on the binomial distribution. Differences in the proportion of people with a strong response among the six study sites as well as between the current and previous surveys in four of these sites, age groups, gender and commuting status were examined by Fisher's exact test and, in accordance with suggestions made by McNutt *et al.* (2003) and Zou (2004), by multiple Poisson regression with a robust estimator of variance yielding adjusted prevalence ratios (PR). We also used censored normal regression applied to log-transformed data to analyse the difference in measured response to antigens between the current and previous studies. The results are presented as a ratio of the geometric mean from the present study to that from the previous one. All statistical tests were evaluated as two-sided at a significance level of 0.05. Statistical analysis was performed by the statistical software Stata, release 9.2 (Stata Corp. LP, College Station, TX, USA).

RESULTS

Seroprevalence in 2004–2005

Eighty per cent of all persons surveyed had a detectable response to the 15/17-kDa antigen and 94% had a detectable response to the 27-kDa antigen. One person had an undetectable response to the 27-kDa antigen but a detectable response to the 15/17-kDa antigen. However, 44 persons had an undetectable response to the 15/17-kDa antigen but a detectable response to the 27-kDa antigen. The lowest detectable responses were found in site D (river-bank infiltration with partial treatment) where 67% and 84% of persons had a response to the 15/17-kDa and 27-kDa antigen, respectively. In all of the other sites, the detectable responses ranged from 80 to 84% for the 15/17-kDa antigen and 92 to 98% for the 27-kDa antigen.

Sixty-seven per cent of all persons had a strong response to the 15/17-kDa antigen, and 88% had a strong response to the 27-kDa antigen (Table 2). The respective median responses to the 15/17-kDa and 27-kDa antigens were 40% and 61% of PC. For each site, the median responses ranged from 20 to 59% of PC for the 15/17-kDa antigen and 37 to 84% of the PC for the 27-kDa antigen.

We found statistically significant differences among the study sites in the probability of persons having a strong response to the 15/17-kDa antigen ($p = 0.006$) in a Poisson regression analysis after adjustment for age, gender and commuting status (Table 3). The statistically significant difference in the prevalence of a person having a strong response for the 15/17-kDa antigen between site D and F is almost as large as the statistically significant difference found between site D and sites A and B (surface waters with conventional filtration). In comparison with site D, the prevalence of persons having a strong response for the 15/17-kDa antigen is more than 1.5 times higher in sites F (PR = 1.55), B (PR = 1.61) and A (PR = 1.68) holding all other variables in the model at a fixed value. The prevalence for persons in sites C (groundwater from a fissured sandstone aquifer) and E (groundwater from a chalk aquifer)

Table 3 | Associations of a strong serological response (≥20% of PC) with study site, age group, gender and commuting (2004–2005)

	15/17-kDa			27-kDa		
	Adjusted prevalence ratio[a]	**95% CI**	***p*-value**	**Adjusted prevalence ratio[a]**	**95% CI**	***p*-value**
Site			**0.006**			**0.095**
D (BF)	1.00			1.00		
F (BF)	1.55	1.10–2.17	0.012	1.14	0.92–1.40	0.222
E (GW)	1.23	0.87–1.74	0.244	1.23	1.03–1.48	0.023
C (GW)	1.32	0.93–1.88	0.122	1.26	1.05–1.51	0.014
B (SW)	1.61	1.17–2.21	0.003	1.28	1.07–1.53	0.008
A (SW)	1.68	1.23–2.30	0.001	1.28	1.06–1.54	0.010
Age group			**0.001**			**0.018**
18–29	1.00			1.00		
30–39	1.25	1.01–1.54	0.037	1.11	1.00–1.24	0.053
40–57	1.43	1.18–1.74	<0.001	1.16	1.05–1.28	0.005
Gender			**0.468**			**0.807**
Male	1.00			1.00		
Female	1.06	0.91–1.24	0.468	1.01	0.93–1.10	0.807
Commuting			**0.321**			**0.975**
No	1.00					
Yes	0.91	0.76–1.10	0.321	1.00	0.91–1.10	0.975

PC, positive control; SW, surface water; GW, groundwater; BF, river-bank infiltration; 95% CI, 95% confidence interval.
[a]Prevalence ratio for a particular factor is adjusted for all other factors included in the table.

having a strong response for the 15/17-kDa antigen was not statistically different than site D.

Although, some differences in prevalence for persons with a strong response to 27-kDa antigen can be seen among the sites (lower prevalence in sites D and F compared to the other four sites), the site factor was not statistically significant ($p = 0.095$) in a Poisson regression analysis after adjustment for age group, gender and commuting status (Table 3).

The prevalence of a strong response for both antigens also significantly increased with age, but there were no statistically significant differences in a person's strong response associated with gender or commuting status (Table 3).

The Poisson regression analysis also considered the aggregation of sites according to types of water source (i.e., surface water, groundwater and river-bank infiltration). Statistically significant differences among types of water sources were found in the prevalence of persons with a strong response to both the 15/17-kDa ($p = 0.003$) and the 27-kDa antigen ($p = 0.012$). The prevalence of a strong response to the 15/17-kD antigen was similar for persons using river-bank filtered water and groundwater, but significantly higher for persons using surface water (PR = 1.31; 95% CI = 1.08–1.58). For the 27-kDa antigen, a strong response was much more likely for persons using groundwater or surface water than for persons using river-bank filtered water. For groundwater users, the prevalence of a strong response was higher by 17% (PR = 1.17; 95% CI = 1.04–1.31) and for surface water users the prevalence of a strong response was higher by 20% (PR = 1.20; 95% CI = 1.06–1.35). These prevalence ratios are adjusted for age, gender and commuting. More details for this analysis are shown in Table S1, Supplementary Material, available with the online version of this paper.

Comparison of seroprevalence in 2004–2005 and 1985

Sites A–D were included in both surveys. We found that 77% of the 2004–2005 survey participants in these four sites had a detectable response for the 15/17-kDa antigen and 91% had a detectable response for the 27-kDa antigen. These percentages are nearly the same as we found in the 1985 survey ($p = 0.970$

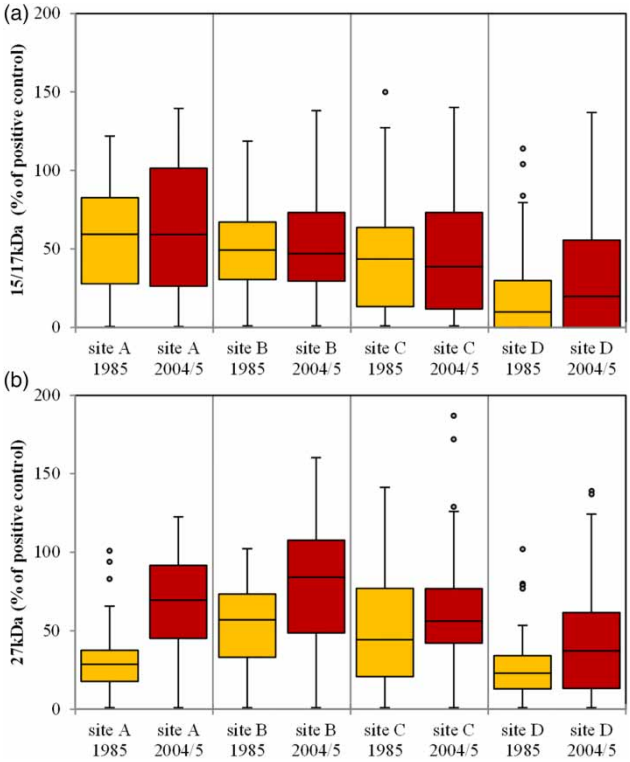

Figure 2 | Intensity of antigen responses in the four sites included in both surveys for 15/17-kDa (a) and 27-kDa (b). Two extremely high values (261% and 501%) in site D for 27-kDa in 2004–2005 are not shown.

and $p = 0.415$, respectively, for each antigen). Neither the intensity of response to 15/17-kDa antigens (Figure 2(a), Table 4, censored normal regression) nor the prevalence of a strong response (Table 4, Poisson regression) were significantly different between the 1985 and 2004–2005 surveys. However, the situation with responses to the 27-kDa antigen is quite different. There are significant differences both in the intensity (Figure 2(b), Table 4) and the proportion of people with the strong response (Table 4). Using the 1985 study as a baseline, geometric means ratios and prevalence ratios indicate a substantial increase in response in the 2004–2005 study, especially in site A.

DISCUSSION

We found a statistically significant elevated serological response for 27-kDa antigens in the 2004–2005 survey compared to the 1985 survey. If drinking water was the primary source of exposure to *Cryptosporidium*, we should have found the opposite, since wastewater and water treatment efficiencies have improved and bottled water consumption has increased in the Czech Republic over the past 20

Table 4 | Comparison of current (2004–2005) and previous (1985) results for each of the four sites included in both surveys

		15/17-kDa			27-kDa		
Site	**N**	**GM ratio[b]**	**95% CI**	***p*-value**	**GM ratio[b]**	**95% CI**	***p*-value**
Censored normal regression[a]							
All sites	399	0.94	0.64–1.37	0.741	1.52	1.22–1.91	<0.001
A (SW)	99	0.75	0.42–1.35	0.339	1.80	1.19–2.73	0.006
B (SW)	100	0.68	0.36–1.31	0.249	1.43	1.04–1.98	0.029
C (GW)	100	1.01	0.45–2.28	0.978	1.46	0.99–2.15	0.055
D (BF)	100	1.61	0.56–4.65	0.377	1.39	0.72–2.69	0.318
Site	**N**	**Prevalence ratio[b]**	**95% CI**	***p*-value**	**Prevalence ratio[b]**	**95% CI**	***p*-value**
Poisson regression[c]							
All sites	399	0.97	0.85–1.10	0.646	1.20	1.09–1.32	<0.001
A (SW)	99	0.97	0.80–1.19	0.804	1.36	1.11–1.67	0.003
B (SW)	100	0.90	0.75–1.08	0.255	1.05	0.94–1.17	0.402
C (GW)	100	0.83	0.63–1.08	0.165	1.19	1.00–1.41	0.049
D (BF)	100	1.38	0.86–2.23	0.184	1.25	0.93–1.67	0.136

PC, positive control; SW, surface water; GW, groundwater; BF, river-bank infiltration; 95% CI, 95% confidence interval; GM ratio, geometric means ratio.
[a]Differences in geometric means of measured response.
[b]Results from the 2004–2005 survey relative to the 1985 survey, adjusted for age group and gender.
[c]Differences in a prevalence of persons with a strong response (≥20% of PC).

years. The amount of untreated waste water has decreased from 277 mil. m^3 in 1992 to 30 mil. m^3 in 2004, which led to 10 times reduction of emitted pollution expressed as 5-day biochemical oxygen demand, chemical oxygen demand and suspended solids approximately (CENIA 2018). The consumption of nonalcoholic beverages including bottled water was almost three times lower in 1985 than in 2004 (94 vs 275 L per person and year) (Czech Statistical Office 2014). These factors should have reduced drinking water exposures and the seroprevalence.

We also found a strong serological response among a very high percentage of the persons surveyed, and the percentage with a strong response is greater than reported in other countries (Frost *et al.* 2000a, 2003, 2005a; Egorov *et al.* 2004; Tollestrup *et al.* 2014; Farkas *et al.* 2015) (Figure 3). The strong responses were found in both current and previous surveys and in all sites, with the exception of site D (river-bank infiltration with partial treatment). Even persons living in site E with groundwater sources from a deep aquifer without any obvious risk of contamination had a very high response; more than 90% of them had a strong response to the 27-kDa antigen. Combining sites with similar water sources, we found that the prevalence of a strong response to the 15/17-kDa antigen was similar for persons using river-bank filtered water and groundwater, but for persons using surface water, the prevalence of a strong response was much higher. The prevalence of a strong response to the 27-kDa antigen for persons using groundwater or surface water was much higher than for persons using river-bank filtered water. Although significant differences in the probability of a strong response were associated with the type of water source in our current survey, the observed results were consistent with additional transmission pathways in addition to drinking water. What the most important sources of exposure are in the Czech Republic is not clear.

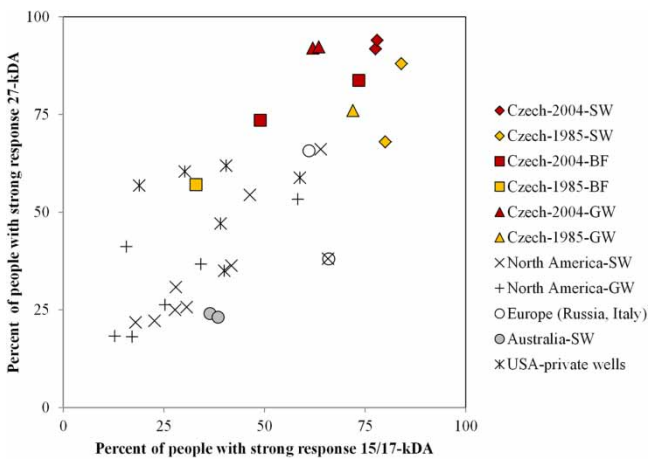

Figure 3 | Strong serological response (≥20% of PC) for 15/17-kDa and 27-kDa *Cryptosporidium* antigens in Czech surveys and surveys in other countries. Sera in North America, Europe, Australia surveys were analysed in the same laboratory as Czech sera (Frost *et al.* 2003; Kozisek *et al.* 2008; Tollestrup *et al.* 2014). SW, surface water; BF, river-bank infiltration; GW, groundwater.

Sporadic cryptosporidiosis risk factors include: contact with animals, contact with another person with diarrhoea (Hunter *et al.* 2004; Roy *et al.* 2004; Pintar *et al.* 2009), swimming in lakes, rivers (Roy *et al.* 2004; Pintar *et al.* 2009) and swimming pools (Egorov *et al.* 2018), consumption of unboiled drinking water (Hunter *et al.* 2004) or water not additionally treated at home (Egorov *et al.* 2018), taking care of children under five years (Hunter *et al.* 2004) and travelling (Hunter *et al.* 2004; Pintar *et al.* 2009). One possible change in exposure that might be responsible for the increased seroprevalence includes increased travel outside the country. Travel abroad has increased dramatically in the past 20 years and imported and secondary infections from travel might be partly responsible for the increased seroprevalence. For example, in the Waterloo district of Canada in 2005–2007 28% of recorded cases of cryptosporidiosis were imported cases (Pintar *et al.* 2009).

Contaminated food can also contribute to an elevated serological response. Consumption of raw vegetables has been involved in several foodborne outbreaks of cryptosporidiosis (Ryan *et al.* 2018). However, the consumption of raw vegetables is also recognized to have a protective effect on cryptosporidiosis (Hunter *et al.* 2004; Roy *et al.* 2004), and increased consumption may be associated with an increased seroprevalence of antigens and protection against symptoms. A change in this exposure for *Cryptosporidium* is supported by reports of increased consumption of vegetables and fresh salads in the Czech Republic. The Czech Statistical Office found that the consumption of vegetables increased from 68.1 to 79.8 kg per year per person from 1985 to 2004; the consumption of cucumbers, tomatoes and peppers, which usually are consumed fresh, more than doubled (Czech Statistical Office 2008), but the consumption of lettuce, which was involved in some foodborne outbreaks of cryptosporidiosis (Ryan *et al.* 2018), remained unchanged (Czech Statistical Office 2008).

Another factor to consider is the discrepancy between the high seroprevalence and the low number of cases of cryptosporidiosis reported in the Czech Republic. Although cryptosporidiosis has been included among the compulsorily reported diseases since the early 1980s, the number of reported cases of cryptosporidiosis each year has always been less than 10, and sometimes no cases have been reported. Although this may not reflect the real situation, the extent of under-reporting is not well known. The ability of Czech clinical labs to analyse stool samples for *Cryptosporidium* oocysts is quite good, as the national proficiency testing programmes have been organized since 2002 (Tolarová 2002). However, not all stool samples submitted for parasitological analysis are analysed for *Cryptosporidium* oocysts. According to data from the National Reference Laboratory for Intestinal Parasites, only some 3% of stool samples tested for *Giardia* are tested for *Cryptosporidium*. Statistics from the study of diarrhoea among children in one regional hospital in South Bohemia suggest the extent to which cases may be under-reported. In this study, *Cryptosporidium* oocysts were detected in 11% of children (2–36 months of age) admitted to the hospital for diarrhoea between 1992 and 1996 (Chmelík *et al.* 1998). The situation may be different for persons older than three years. For example, in a regional hospital located in site B, none of the stool samples from 378 patients with diarrhoea (age 3–66 years) taken between 1997 and 2002 were found positive for oocysts of *Cryptosporidium* spp. (Fajfrlík, written communication, 13 January 2013). The low numbers of reported cryptosporidiosis cases, which is similar to other Central and Eastern European countries (Plutzer *et al.* 2018), may be due not only to under-reporting but also to protective immunity (Frost *et al.* 2005b). The significantly increased seroprevalence and large percentage of strong responses for the 27-kDa antigen may be, in part, a reflection of chronic, low-level exposures that have provided protective immunity among older children and adults. It is also possible that the infections occurred many years earlier, but the response had not waned sufficiently. There is some evidence of higher responses with each progressive infection (Priest *et al.* 2006).

There are other factors which may affect the interpretation of our results. While the more recent sera from 2004 to 2005 were collected in December and January, the older sera (1985) were collected in September and October. This may possibly have affected exposure patterns among the two surveys. Persons surveyed in the autumn may not have yet had time to develop a proper serological response if exposure occurred to swimming. The prevalence of human cryptosporidiosis is not distributed homogenously during the year. In the United States, the summer and early autumn peak of cases, which was probably caused by swimming and other outdoor activities, was clearly visible (Yoder & Beach 2010). A similar increase of cases of cryptosporidiosis was noticed in England and Wales where higher autumn peak of cases was caused by *C. hominis*, the lesser spring peak by *C. parvum* (Sopwith *et al.* 2005). Moreover, the autumn peak was reported from other European countries (Germany, Sweden, Spain) (Semenza & Nichols 2007). If we assume the same seasonal distribution in Central Europe and that not all infections may be symptomatic, a small part of the differences between sera prevalences from 1985 and 2004 may be due to the slightly different sampling months. If seasonal differences reflect exposure from swimming, the most important bias would be for younger children and teens, who were not included in our survey.

Another factor is the source of sera. The sera in the Serum Bank of the National Institute of Public Health, which were used in our previous study, were probably better representative for the general population. The sera in the 2004–2005 study came from regular blood donors, i.e., persons who are healthier and have also exhibited a healthier lifestyle than the average of the general adult population (Atsma *et al.* 2011). However, it is questionable how the sera donor differences can significantly influence seroprevalence.

We can also rule out systematic laboratory error since all serum samples were analysed in the same laboratory by the same experienced analyst.

CONCLUSIONS

We hypothesized a lower seroprevalence of *Cryptosporidium* antigens because of improved management of wastewater and drinking water and increased bottled water consumption in the Czech Republic. On the contrary, we found a higher seroprevalence and a higher percentage of persons with a strong serological response. In addition, results of our survey and the few reported cases of cryptosporidiosis in the Czech Republic suggest that chronic, low-level exposures

to *Cryptosporidium* may be affording protection against symptoms and illness. If so, an issue that must be considered is the possible negative aspects of such immunity. For immunocompetent persons, development of protective immunity leads to fewer episodes of symptomatic cryptosporidiosis. However, people who are immunosuppressed, due to any of a variety of diseases, may be at elevated risk of severe, if not lethal, *Cryptosporidium* infections, and this must be considered in areas with oocyst contaminated drinking water. This survey has helped provide a better understanding of the epidemiology of *Cryptosporidium* infections in the Czech Republic and emphasizes the importance of additional research to clarify the various sources of exposure, especially waterborne.

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