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Risk Factors for Sporadic Cryptosporidiosis in the Netherlands: Analysis of a 3-Year Population Based Case-Control Study Coupled With Genotyping, 2013–2016

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Background. In 2012, cryptosporidiosis cases increased in the Netherlands, but no single source was identified. In April 2013, we began a 3-year population-based case-control study coupled with genotyping to identify risk factors for sporadic cryptosporidiosis.

Methods. *Cryptosporidium* cases were laboratory confirmed (by microscopy or polymerase chain reaction), and the species (ie, *C. hominis* or *C. parvum*) was determined. We analyzed data by study year, combined and by species. We performed single-variable analysis, and variables with a *P* value of ≤ .10 were included in a multivariable logistic regression model adjusting for age, sex, and season.

Results. The study included 609 cases and 1548 frequency-matched controls. *C. parvum* was the predominant species in the first 2 study years, shifting to *C. hominis* in the third year. Household person-to-person transmission and eating barbequed food were strongly associated with being a case. Eating tomatoes was negatively associated. When the analysis was stratified by study year, person-to-person transmission was an independent risk factor. Analysis by species identified different risk factors for cases infected with *C. parvum* and *C. hominis*.

Conclusion. This was the first case-control study examining risk factors for sporadic cryptosporidiosis in the Netherlands. Providing information about *Cryptosporidium* exposure during outdoor activities and improvements in hygiene within households could prevent future sporadic infections.

Keywords. *Cryptosporidium*; sporadic cryptosporidiosis; case-control study; risk factors; the Netherlands.

Cryptosporidiosis is a diarrheal disease caused by protozoa of the genus *Cryptosporidium*. Most human infections are caused by 2 species: *C. hominis,* which is reportedly restricted to humans, and *C. parvum,* which can also be found in a wide range of animals [1–4]. The incubation period varies from 1 to 12 days (average, approximately 7 days), and symptoms in immunocompetent patients can continue for up to 4 weeks [3, 5]. The most common symptom of cryptosporidiosis is watery diarrhea, but abdominal pain, fever, nausea, and dehydration can also occur. Transmission occurs via the fecal-oral route, via person-to-person or animal-to-person spread, or indirectly by either consumption of contaminated drinking water or water during recreational activities or eating contaminated food [2, 6].

In the western hemisphere, it is estimated that ≥90% of cryptosporidiosis cases occur sporadically [7]. However, most information about cryptosporidiosis has been gathered from outbreak investigations, which have found cryptosporidiosis to be associated with water from public and private supplies, swimming pools, unpasteurized milk, and contact with farm animals, especially during farm visits [8–13].

There are several studies from the early 2000s in the literature detailing the epidemiology and etiology of sporadic cryptosporidiosis [7, 14]. In the Netherlands, the number of *Cryptosporidiu*m cases in previous studies to establish the etiology of gastroenteritis in sentinel general practices was too low to identify risk factors [15, 16]. *Cryptosporidium* infection is not a notifiable disease in the Netherlands, and no enhanced surveillance program exists. In addition, diagnostic assays for *Cryptosporidium* are only performed when specifically requested by the referring physician. Thus, the incidence of human cryptosporidiosis is poorly known. In the summer of 2012, the Netherlands experienced a large increase in cryptosporidiosis cases, with >520 positive samples reported by laboratories throughout country. An epidemiological investigation was conducted, but no single source was identified [17].

In April 2013, we began a large, 3-year, population-based case-control study. The study was designed to describe the epidemiology of sporadic *Cryptosporidium* infections and to identify risk factors for sporadic clinical infections with *Cryptosporidium* in the Netherlands, to acquire information for control strategies.

METHODS

Recruitment of Cases and Controls

Through collaboration with 17 regional medical microbiological laboratories that perform *Cryptosporidium* detection in the Netherlands, we recruited *Cryptosporidium*-positive cases into our study and obtained *Cryptosporidium*-positive fecal samples and/or extracted DNA. Recruitment began 1 April 2013 and lasted until 30 March 2016.

Recruitment of study participants involved coordination between the collaborating laboratories and general practitioners (GPs) of patients with recently diagnosed *Cryptosporidium* positivity (Figure 1). The collaborating laboratories also sent positive *Cryptosporidium* fecal samples and/or extracted DNA to the National Institute for Public Health and the Environment (RIVM) Center for Infectious Disease Research, Diagnosis, and Screening for further identification and typing of the species. These samples were coded with the same unique identifier as the written material sent to patients. Therefore, the typing

results could be linked to the case questionnaire. Fecal samples or extracted DNA were only typed after receiving the patient questionnaire (Figure 1).

Controls were prospectively selected from the population register by frequency matching for age and area of residence [18]. The expected number of cases, stratified by age, was calculated using historical data on cryptosporidiosis, recorded during 2010–2012, from the laboratories. The controls each received a letter explaining the study rationale and objectives, the control questionnaire, and a prepaid envelope to return the questionnaire and informed consent to the RIVM.

Genotyping of *Cryptosporidium*-Positive Samples

Cryptosporidium species were determined using real-time polymerase chain reaction (PCR) analysis incorporating specific primers and probes for a *C. parvum* hypothetical protein and a *C. hominis* target for part of the GP60 gene [19]. These specific markers were chosen because *C. parvum* and *C. hominis* had been previously detected in the Netherlands during the 2012 increase in *Cryptosporidium* infections [17], and our study aimed to identify sporadic cases for cryptosporidiosis in the Netherlands. The duplex real-time PCR used is able to amplify a specific *C. parvum* sequence and a specific *C. hominis* sequence, allowing detection of coinfection with both species.

Case and Control Definitions

We defined a cryptosporidiosis case as occurrence of gastrointestinal illness within 2 weeks of laboratory confirmation (by microscopy or PCR) of *Cryptosporidium* infection. Because we were examining for risk factors for sporadic cryptosporidiosis within the Netherlands, study participants with a travel history outside of the Netherlands in the 2 weeks prior to symptom onset (cases) or completion of the questionnaire (controls) were excluded from our analysis. Controls were excluded if they had diarrheal illness 2–4 weeks before completing the questionnaire.

Data Collection

Both cases and controls received a paper-based questionnaire for self-administration. For children aged <16 years, the primary caretaker was requested to complete the questionnaire. Study participants were asked to complete questions relating to demographic and household information, symptoms of illness, medical history, foreign travel, contact with animals (pets and farm animals), contact with ill persons, daily and recreational activities, and food and drink consumption. In addition, cases were requested to provide their email addresses for participation in a follow-up study regarding the long-term sequelae of cryptosporidiosis (ie, sequelae 4 months after disease onset). The questions referred to the 14-day period before onset of symptoms, for cases, and the 14-day period before completion of the questionnaire, for controls. Paper questionnaires returned to the RIVM were entered into a password-protected Access database (Microsoft; Redmond, WA).
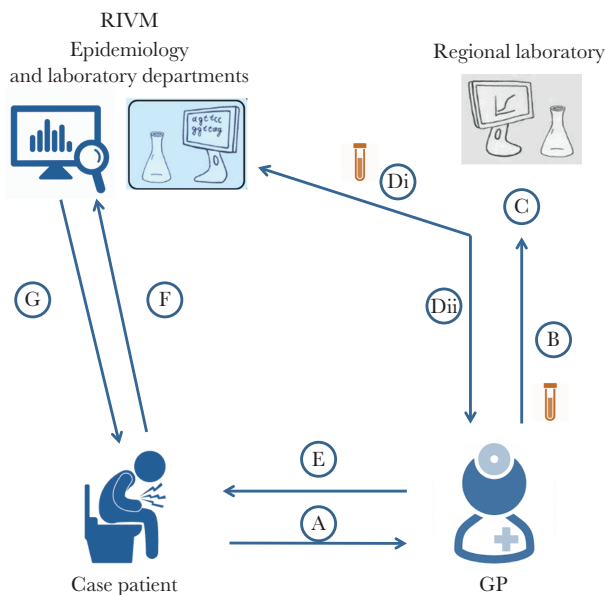

Figure 1. Recruitment of *Cryptosporidium*-positive cases into the study, April 2013–March 2016, the Netherlands. *A*, Patient with gastrointestinal symptoms visits general practitioner (GP). *B*, GP sends a fecal sample to regional laboratory. *C*, Regional laboratory performs tests. *D*, If sample is *Cryptosporidium* positive, laboratory sent sample for further speciation/typing to National Institute for Public Health and the Environment laboratory (*i*) and sent envelope to GP to recruit case (*ii*). *E*, GP receives information pack and forwards it to case. *F*, Case returns questionnaire to RIVM. *G*, Cases that provided their email for follow-up study were invited to complete follow-up questionnaire 4 months later.

Data Analysis

Analyses were performed using cross-tabulations, χ^2 tests, and logistic regression models for significance testing of >250 variables. Odds ratios (ORs) and adjusted ORs (aORs) were calculated with 95% confidence intervals (CIs) and 2-tailed *P* values. We analyzed data by each study year and also conducted a combined analysis of all study years. In addition, analyses were conducted on cases for whom genotyping revealed *C. parvum* or *C. hominis* to be the infecting species. Single-variable analysis was performed, adjusting for age, sex, and season, to assess the possible association between various exposures and cryptosporidiosis. To identify independent risk factors for cryptosporidiosis, variables found to be positively or negatively associated with illness ($P \leq .10$) were included in a multivariable logistic regression model adjusted for age, sex, and season. A manual backward selection procedure was used, and variables with a *P* value of $\leq .05$ following likelihood ratio testing were retained in the final multivariable model. Where there were too many variables to add to the model, non-significant variables with the most missing data were removed. Multivariable submodels were also developed, and significant variables were combined in the final multivariable model.

Ethics Statement

This study received ethical approval from the Medical Research Ethics Committee of Utrecht University (protocol 13–145/C). No personal information was analyzed as part of this study. Study participants who provided their email addresses for future follow up had their addresses stored separately.

RESULTS

Between April 2013 and March 2016, 1860 cases and 7081 controls were invited to participate in the study. Of those, 609 cases (32%) and 1548 controls (22%) were included in our analysis (Figure 2). The median age was 26 years (range, 0–95 years) among cases and 27 years (range, 0–91 years) among controls. Female participants accounted for 59% of cases and 58% of controls (Figure 3). Overall, 427 *Cryptosporidium* samples were speciated, of which 304 were *C. parvum* (59%), 123 were *C. hominis* (24%), and 84 (16%) had no detectable PCR signal for either species (Figure 4). We found that the number of *Cryptosporidium* infections increased in late summer in the first 2 study years, while in the third study year, the number of infections peaked in late summer and remained high until early winter.

Approximately 80% or more of cases reported diarrhea, abdominal pain, loss of appetite, and fatigue. The median diarrhea duration was 13 days (range, 1–64 days). One third or more of cases also reported vomiting, weight loss, and dizziness. Over the 3 study years, 38 cases were admitted to hospital for up to 19 days (Table 1).

In the first year of the study, we could determine the species for 149 cases; 135 (91%) had *C. parvum* infection, and 14 (9%) had *C. hominis* infection. We detected 19 cases who had no signal for either species. We included 187 cases (response rate, 52%; female sex, 60%; median age, 22 years [range, 0–95 years]) and 581 controls (response rate, 32%; female sex, 56%; median age, 21 years [range, 0–84 years]) (Figure 3). Results from the final multivariable model showed that cases were more likely than controls to take immunosuppressant medication (aOR, 5.2; 95% CI, 2.5–11), to have had a picnic or barbeque (aOR, 2.8; 95% CI, 1.7–4.5), to have had household person-to-person transmission (aOR, 2.0; 95% CI, 1.2–3.6), or to have swum in an inflatable pool (aOR, 1.8; 95% CI, 1.0–3.0). Cases were less likely to play in a sandbox (aOR, 0.5; 95% CI, .3–.8) or eat tomatoes (aOR, 0.6; 95% CI, .4–.9), compared with controls (Table 2).

In the second year of the study, we could determine the species for 132 cases; 117 (92%) had *C. parvum* infection, and 15 (8%) had *C. hominis* infection. We detected 13 cases who had no signal for either species. We included 159 cases (response
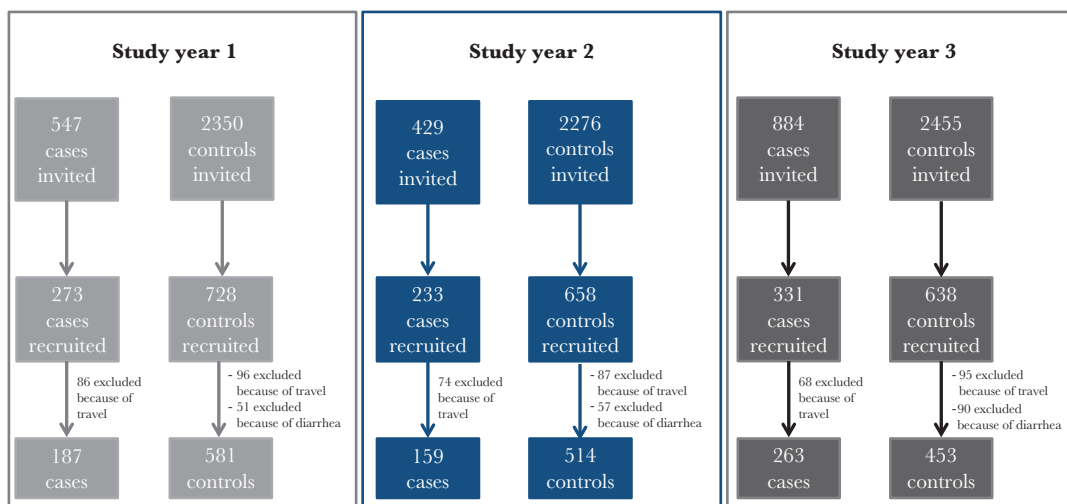

Figure 2. Number of cases and controls recruited into the case-control study, April 2013–March 2016, the Netherlands.
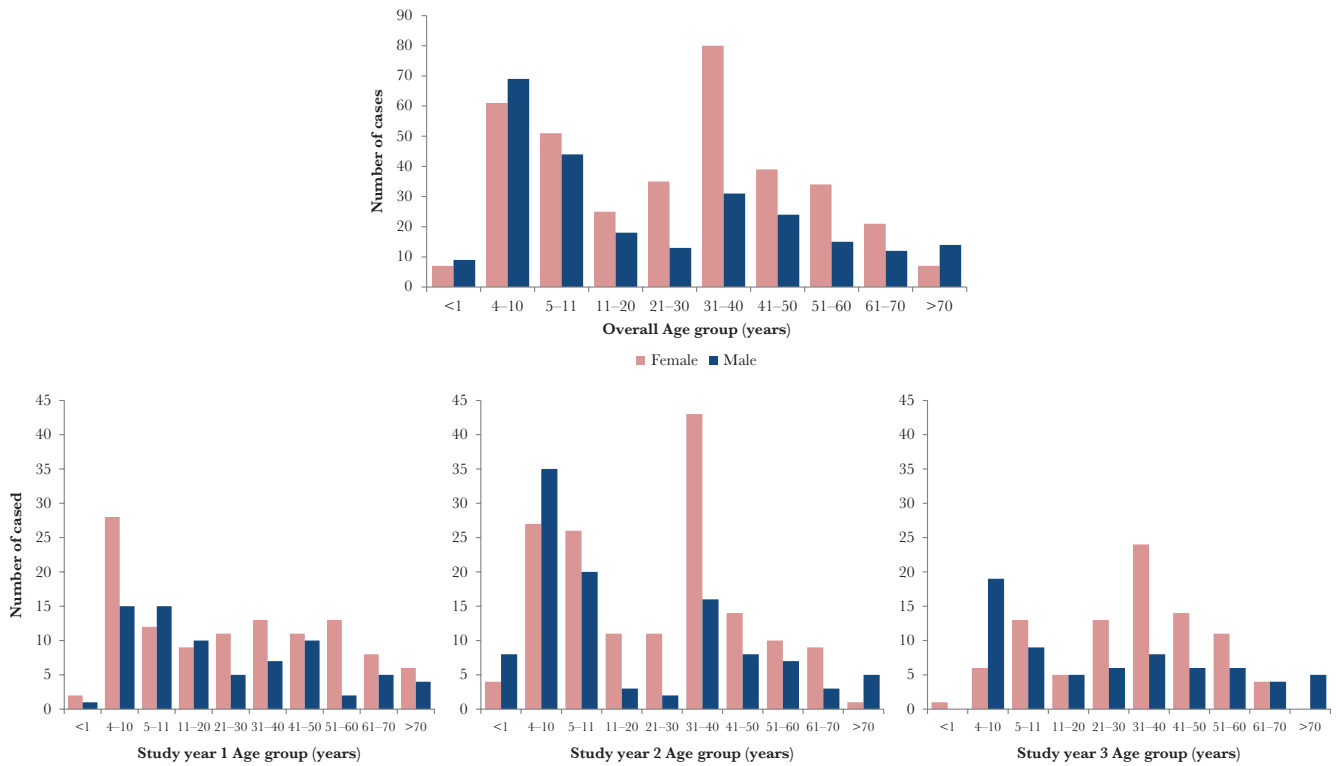
Figure 3. Age distribution and sex of cases, by study year, April 2013–March 2016, the Netherlands.

rate, 54%; female sex, 57%; median age, 30 years [range, 0–92 years]) and 512 controls (response rate, 29%; female sex, 58%; median age, 30 years [range, 0–91 years]) (Figure 3). In

the final multivariable model, we found that cases were more likely than controls to have had nonhousehold person-to-person transmission (aOR, 3.4; 95% CI, 1.3–9) or to have swum in
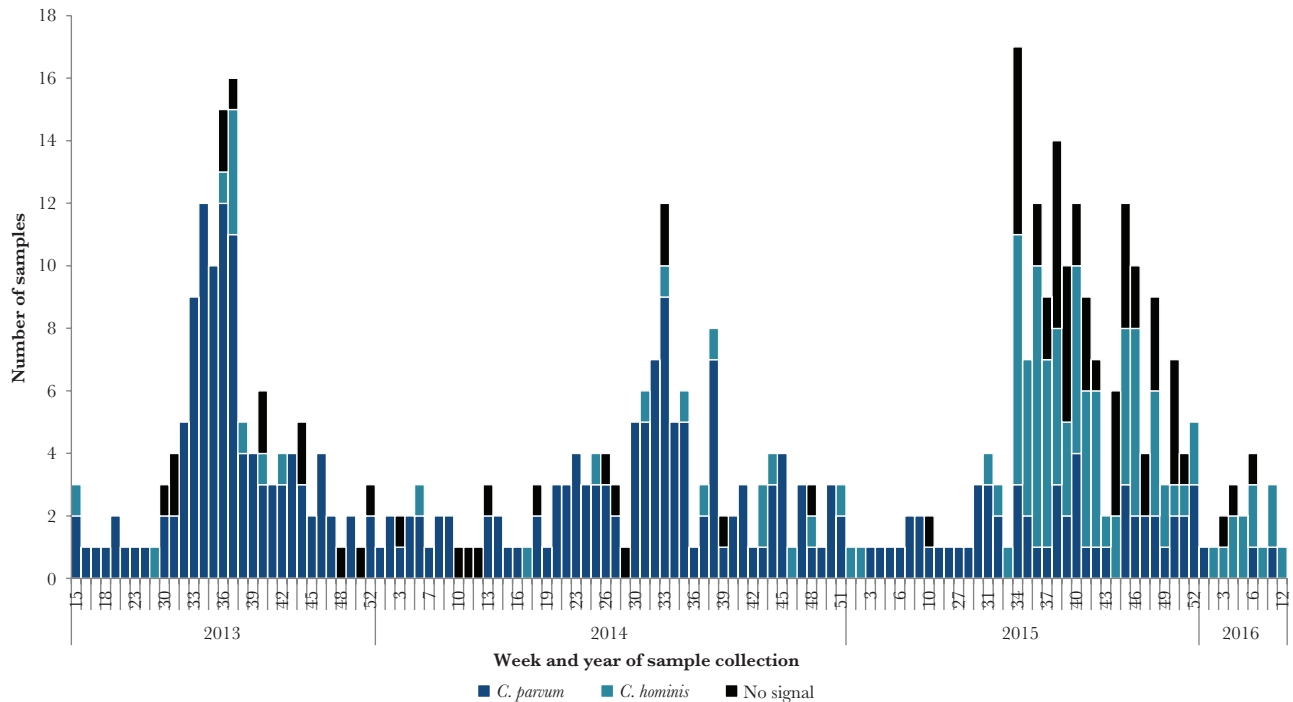

Figure 4. Distribution of *Cryptosporidium* species among 511 cases with completed questionnaires, April 2013–March 2016, the Netherlands.

Table 1.   Overall Reported Clinical Symptoms Among Cases of Cryptosporidiosis, by Species Type and Study Year, April 2013–March 2016, the Netherlands

Clinical symptoms	Overall	*C. parvum*	*C. hominis*	Study Year 1	Study Year 2	Study Year 3
Diarrhea	520 (87.0)	264 (87.4)	103 (85.8)	166 (89.7)	132 (85.2)	222 (86.1)
Reported continued diarrhea	111 (21.9)	42 (16.5)	34 (32.7)	34 (20.9)	26 (20.5)	51 (23.6)
Diarrhea duration, d	13 (1–64)	12 (0–64)	16 (1–56)	13 (2–64)	12 (1–51)	15 (1–56)
Abdominal pain	484 (84.3)	242 (83.2)	99 (87.6)	143 (81.3)	127 (83.0)	214 (87.3)
Vomiting	193 (36.4)	101 (37.0)	36 (34.0)	52 (32.3)	55 (38.5)	86 (38.0)
Loss of appetite	454 (79.4)	231 (80.2)	92 (80.0)	139 (79.0)	119 (78.8)	196 (80.0)
Weight loss	356 (65.7)	198 (72.3)	63 (57.3)	109 (66.1)	102 (70.8)	145 (62.2)
Headache	204 (40.8)	102 (40.0)	44 (44.4)	51 (33.6)	63 (45.7)	90 (42.9)
Fatigue	455 (81.1)	238 (83.2)	89 (78.8)	142 (83.5)	117 (78.5)	196 (81.0)
Dizziness	155 (32.4)	77 (31.3)	32 (34.0)	48 (32.7)	42 (32.5)	65 (32.0)
Eye pain	67 (14.0)	37 (15.0)	10 (10.5)	20 (13.5)	25 (19.2)	22 (11.0)
Joint pain	118 (24.6)	61 (24.6)	24 (25.5)	39 (26.4)	32 (24.6)	47 (23.3)
Hospitalized	38 (6.3)	25 (8.3)	10 (8.1)	9 (4.8)	15 (9.7)	14 (5.3)
Hospitalization duration, d	4 (0–19)	3 (0–19)	3 (0–11)	6 (4–8)	3 (0–19)	5 (0–15)

Data are no. (%) of cases or median value (range).

an inflatable pool (aOR, 2.6; 95% CI, 1.2–5.8). Cases were less likely to have eaten chicken, compared with controls (aOR, 0.6; 95% CI, .4–.9) in the 2 weeks prior to completing the questionnaire (Table 2).

In the third year of the study, we could determine the species for 146 cases; 94 (64%) had *C. hominis* infection, and 52 (36%)

Table 2.   Risk Factors for Cryptosporidiosis, Overall and by Study Year, According to Final Multivariable Models, April 2013–March 2016, the Netherlands

Risk Factor	aOR[a] (95% CI)	P
Overall[b]		
Household person-to-person transmission	2.2 (1.7–3.0)	<.001
Ate barbequed food	1.8 (1.4–2.3)	<.001
Ate tomatoes	0.6 (.5–.8)	<.001
Study year 1[c]		
Taking immunosuppressant medication	5.2 (2.5–11)	<.001
Having picnic or barbeque	2.8 (1.7–4.5)	<.001
Household person-to-person transmission	2.0 (1.2–3.6)	.011
Swam in an inflatable pool	1.8 (1.0–3.0)	.033
Playing in a sandbox	0.5 (.3–.8)	.004
Eat tomatoes	0.6 (.4–.9)	.016
Study year 2[d]		
Nonhousehold person-to-person transmission	3.4 (1.3–8.8)	.011
Swam in an inflatable pool	2.6 (1.2–5.8)	.018
Ate chicken	0.6 (.4–.9)	.009
Study year 3[e]		
Nonhousehold person-to-person transmission	6.0 (2.5–14)	<.001
Household person-to-person transmission	5.0 (2.1–9.5)	<.001
Contact with animal feces	2.8 (1.3–6.0)	.006
Ate salad	0.6 (.4–.8)	.006
Ate hard cheese	0.7 (.5–.9)	.006

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval.

[a]Adjusted by age, sex, and season.

[b]Based on 1826 observations.

[c]Based on 626 observations.

[d]Based on 414 observations.

[e]Based on 339 observations.

had *C. parvum* infection. We detected 52 cases who had no signal for either species. We included 263 cases (response rate, 54%; female sex, 59%; median age, 18 years [range, 0–81 years]) and 453 controls (response rate, 29%; female sex, 58%; median age, 27 years [range, 0–87 years]) (Figure 3). In the final multivariable model, we found that cases were more likely than controls to have had nonhousehold person-to-person transmission (aOR, 6.0; 95% CI, 2.5–14), to have had household person-to-person transmission (aOR, 5.0; 95% CI, 2.1–9.5), or to have had contact with animal feces (aOR, 2.8; 95% CI, 1.3–6.0). Compared with controls, cases were less likely to have eaten salad (aOR, 0.6; 95% CI, .4–.8) or to have eaten hard cheese (aOR, 0.7; 95% CI, .5–.9) in the 2 weeks prior to completing the questionnaire (Table 2).

Overall results from the combined study years final multivariable model showed that cases were more likely than controls to have had household person-to-person transmission (aOR, 2.2; 95% CI, 1.7–3.0) and to have eaten barbequed meat (aOR, 1.8; 95% CI, 1.4–2.3). Cases were less likely to have eaten tomatoes, compared with controls (aOR, 0.6; 95% CI, .5–.8; Table 2).

Risk Factors for *C. parvum* Infection

In the final multivariable model, we found that cases were more likely than controls to be taking immunosuppressant medication (aOR, 3.2; 95% CI, 1.5–6.7) and to have visited a farm (aOR, 2.5; 95% CI, 1.5–4.3). We also found that those with a higher frequency of consuming water from sources other than taps had greater odds of being a case (aOR, 1.2; 95% CI, 1.0–1.3). Compared with controls, cases were less likely to have eaten chicken (aOR, 0.3; 95% CI, .2–.6), to have eaten carrots (aOR, 0.6; 95% CI, .3–.9), or to live in a larger household (aOR, 0.7; 95% CI, .6–.9; Table 3).

Risk Factors for *C. hominis* Infection

In the final multivariable model, we found that cases were more likely than controls to have had nonhousehold person-to-person

Table 3. Risk Factors for Cryptosporidiosis, by Infecting *Cryptosporidium* Genotype, According to Final Multivariable Models, April 2013–March 2016, the Netherlands

Risk Factor	aOR[a] (95% CI)	P
C. parvum [b]		
Taking immunosuppressant medication	3.2 (1.5–6.7)	.002
Visited a farm	2.5 (1.5–4.3)	<.001
Per dose of other water source consumed[c]	1.2 (1.0–1.3)	.005
Per number of people living in a household	0.7 (.6–.9)	.004
Ate carrots	0.6 (.3–.9)	.028
Ate chicken	0.3 (.2–.6)	.001
C. hominis [d]		
Nonhousehold person-to-person transmission	7.5 (3.2–17.3)	<.001
Swam in an inflatable pool	4.7 (2.4–9.5)	<.001
Household diarrheal case	3.5 (1.7–7.1)	<.001
Per number of people aged >18 years per household	0.4 (.2–.9)	.018

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval.

[a]Adjusted by age, sex, and season.
[b]Based on 715 observations.
[c]Drinking water from sources other than a tap.
[d]Based on 677 observations.

transmission (aOR, 7.5; 95% CI, 3.2–17), to have swum in an inflatable pool (aOR, 4.7; 95% CI, 2.4–9.5), or to have had a household diarrheal case (aOR, 3.5; 95% CI, 1.7–7). Compared with controls, cases were less likely to live in a household with a greater number of people aged ≥18 years (aOR, 0.4; 95% CI, .2–.9; Table 3).

DISCUSSION

This is the first prospective case-control study coupled with genotyping data that investigated risk factors for sporadic cryptosporidiosis in the Netherlands. Through active engagement with 17 regional laboratories, we recruited *Cryptosporidium*-positive cases into our study, which allowed us to conduct analyses by study year, by all study years combined, and of cases for whom genotyping revealed *C. parvum* or *C. hominis* as the infecting species. This enabled us to identify risk factors that may have contributed to sporadic cases of cryptosporidiosis in the Netherlands.

Over the course of this 3-year study, we identified that *C. parvum* was the predominant species in the first 2 years and that, during the third year, a species shift to *C. hominis* occurred. We also found that, in each study year, there was a number of cases without a detectable signal (ie, a high quantitation cycle) for the *C. hominis or C. parvum* markers.

In terms of clinical symptoms between the cases for whom genotyping data were available, we found that the median duration of diarrhea was longer for *C. hominis*–infected cases as compared to *C. parvum*–infected cases. This finding is consistent with a previous case-control study that hypothesized that *C. hominis* infection may be more prolonged than *C. parvum* infection [7]. In addition, the authors of that study suggested

that asymptomatic carriage of *C. hominis* may be more common. However, based on our findings, it suggests that asymptomatic carriage could be common for both *C. parvum* and *C. hominis,* as not all cases reported diarrheal illness. This hypothesis is supported by a recent study in daycare centers in the Netherlands, which found an increase in *Cryptosporidium* infections, mainly in asymptomatic young children [20].

Our prior hypothesis was that sporadic cases of cryptosporidiosis in the Netherlands would be associated with drinking unboiled tap water at home, recreational activities, contact with animals, and contact with other persons with diarrhea. This hypothesis was based on findings from investigations following an increase in cryptosporidiosis in the Netherlands in 1995 [16] and 2012 [17] and from other outbreak investigations and case-control studies [1–3, 7, 14, 21]. However, in our overall combined analysis of the 3 study years, the main risk factors we identified were contact with a household member who had diarrhea and eating barbequed food. We also found that eating tomatoes was negatively associated with cryptosporidiosis. Analysis stratified by each study year identified that person-to-person transmission from a household member and/or a nonhousehold member was independently associated with cryptosporidiosis. Person-to-person transmission is a well-known risk factor for cryptosporidiosis, considering the route of transmission. Secondary transmission has been known to occur in families and in outbreak settings, as well as in daycare centers and hospitals [2, 3, 8, 14]. Interestingly, we found that, over the 3 study years, a high proportion of children below ten years of age and women aged 31–40 years were cases, suggestive of child to caregiver transmission or vice versa [20].

In each study year and in our overall analysis, we identified risk factors related to outdoor activities, such as eating barbequed food, having a picnic, swimming in an inflatable pool, and having contact with animal feces. These risk factors are both proxies for outdoor exposure and poor hygiene and are somewhat similar to those previously identified [7, 14]. Compared with controls, cases had greater contact with pets and farm animals. However, some variables had too few observations to include in the final multivariable model. In single-variable analysis, the question relating to eating unusual food was significant in each study year. However, following examination of the follow-up question about types of unusual food, the types of unusual food listed were not unusual (eg, olives, marshmallows, and avocados). Therefore, it might be more the perception by the participant of eating an unusual food, rather than having actually eaten an unusual food.

In our species analysis, we found that cases with *C. parvum* genotyped reported a higher frequency of taking immunosuppressant medication. Previous studies have shown that immunocompromised individuals are more susceptible to cryptosporidiosis [5, 22]. However, we did not ask study participants to detail which medical condition they had that required them to

take such medication. Nonetheless, we found it interesting that this risk factor was independently identified among *C. parvum*–infected cases and in the first 2 study years, when *C. parvum* was predominant. Although variables related to water consumption were significant in single-variable analysis, we only found an association with drinking water from water sources other than a tap in the final *C. parvum* multivariable model. This may indicate that drinking tap water in the Netherlands is not a source of cryptosporidiosis. We also found that visiting a farm was an independent risk factor among *C. parvum*–infected cases. Several outbreaks have found that visiting farms was associated with cryptosporidiosis [13, 23, 24]. The high number of patients in whom *C. hominis* or *C. parvum* were not detected, particularly in the third year, was unexpected, as *C. hominis or C. parvum* are considered to be the predominant species in humans and had been detected during the increase in *Cryptosporidium* infections in the Netherlands in 2012 [17].

We found that cases with *C. hominis* genotyped reported increased contact or care of others in single-variable analysis, but there were insufficient observations to include this in the final multivariable model. Nonetheless, risk factors associated with person-to-person transmission were strongly associated with cryptosporidiosis, confirming that *C. hominis* is primarily limited to person-to-person transmission [1, 2, 7]. Swimming in an inflatable pool was found to be independently associated with *C. hominis* and has been previously found to be associated with sporadic cases [14]. This factor further suggests that many cases may have been secondary, following sharing an inflatable pool with an infectious case or asymptomatic case. Interestingly, we found that living in a household with a greater number of people aged ≥18 years was negatively associated with being a case, suggesting that increased *C. hominis* transmission occurs in households with younger children. This finding corroborates the high frequency of contact and care of young children reported by cases.

We found some factors related to food and recreation as protective factors against *Cryptosporidium* infection, such as playing in a sandbox, and eating chicken, salad, tomatoes, and hard cheese. While some studies found that eating chicken, salad, and vegetables were risk factors for cryptosporidiosis [1, 2, 10, 25], other studies found these to have a protective effect, due to the possible immunity achieved through repeated low-dose exposure and asymptomatic infection [7]. However, the quantity of chicken consumed is quite high in the Netherlands [26]. Therefore, this may have occurred because of chance or prevarication bias, as cases may have assumed that chicken was a source of their infection. Finally, the finding that playing in a sandbox was a significant protective factor is biologically plausible owing to immunity following repeated exposure among controls to sand or groundwater contaminated with animal or human feces.

Our study is prone to a number of limitations. Cases in the study are laboratory confirmed; therefore, the proportion of young children and those with health-seeking behavior may have been overestimated, as these patients are more likely to visit their GP [27]. Although the method of determining *Cryptosporidium* species using a duplex real-time PCR has the advantage of being cost efficient and able to detect double infections, the targets used make this PCR much less sensitive than PCR used for detection. This led to a number of samples in our study for which findings could be determined. However, we had selected markers for the predominant *Cryptosporidium* species in humans, and because of the number of samples collected, we refrained from doing an analysis using more-discriminatory typing methods. We restricted our analysis to those who had not recently travelled abroad, which reduced the power of the study. However, this analysis was intended to detect risk factors for sporadic *Cryptosporidium* infection within the Netherlands to inform about prevention measures to reduce infection, and inclusion of participants who traveled may have led to biases in the risk analysis, which would also have lowered the power. Owing to the study design, there is a possibility of selection bias among those who completed and returned the questionnaire, in addition to recall bias and prevarication bias among both cases and controls. Furthermore, because controls were not tested for *Cryptosporidium* and asymptomatic infections can occur [28], some participants with cryptosporidiosis may be included among controls, resulting in misclassification bias. However, to decrease the probability of misclassification, we excluded controls who had diarrheal illness 2–4 weeks before completing the questionnaire.

In conclusion, this case-control study coupled with genotyping was the first in the Netherlands to examine risk factors for sporadic cryptosporidiosis and has provided us with information of public health significance. Our study showed that, over the 3 study years, a shift from *C. parvum* to *C. hominis* occurred. Therefore, in the event of an increase or outbreak of *Cryptosporidium* infection, knowledge about species type would be advantageous for implementing control measures. Our study findings highlighted that education about improved hygiene within households to limit person-to-person transmission is required and that asymptomatic carriers could account for a significant number of secondary cases. In particular, identifying swimming in inflatable pools as an important potential source of cryptosporidiosis highlights that children with diarrhea may be transmitting the parasite to other children or caregivers. This study also found that drinking water is unlikely to be a major cause of sporadic cryptosporidiosis in the Netherlands.

Notes

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