Coxiella burnetii Infection Is Lower in Children than in Adults After Community Exposure

Overlooked Cause of Infrequent Q Fever Reporting in the Young

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Background: Q fever is rarely reported in children/adolescents. Although lower reporting rates are commonly attributed to milder disease and subsequent underdiagnosis in infected children/adolescents, pertinent evidence is scarce. We present data from a large, well-defined single-point source outbreak of Q fever to fill this gap.

Methods: We compared (A) Q fever testing and notification rates in children/adolescents who were 0–19 years of age with those in adults 20+ years of age in October 2009; (B) serological attack rates of acute Q fever in children/adolescents with the rates in adults after on-source exposure on the outbreak farm's premises; (C) incidence of Q fever infection in children/adolescents with that in adults after off-source exposure in the municipality located closest to the farm.

Results: (A) Children/adolescents represented 19.3% (59,404 of 307,348) of the study area population, 12.1% (149 of 1217) of all subjects tested in October 2009 and 4.3% (11 of 253) of notified laboratory-confirmed community cases. (B) Serological attack rate of acute Q fever in children with on-source exposure was 71% (12 of 17), similar to adults [68% (40 of 59)]. (C) Incidence of infection in children/adolescents after community (off-source) exposure was 4.5% (13 of 287) versus 11.0% (12 of 109) in adults (adjusted odds ratio: 0.36; 95% confidence interval: 0.16–0.84; $P = 0.02$). No children/adolescents reported clinical symptoms. Proportion of notified infections was significantly lower in children/adolescents (2.5%) than in adults (10.4%; risk ratio: 0.26; 95% confidence interval: 0.08–0.80, $P = 0.02$).

Conclusion: Notified Q fever was less frequent in children/adolescents than in adults. Although underrecognition contributed to this phenomenon, lower rates of infection in children after community exposure played an unexpected major role. On-source (presumed high-dose) exposure, by contrast, was associated with high serological and clinical attack rates not only in adults but also in children/adolescents. Our findings allow for improved age-specific clinical and public health risk assessment in Q fever outbreaks.

Key Words: Q Fever, children, adolescents, epidemiology, incidence

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Q fever is a bacterial zoonosis caused by *Coxiella burnetii*, which is a mandatorily notifiable disease in the Netherlands and most other countries. Ruminants are considered the most common reservoir. *C. burnetii* is shed in birth material from infected animals and most likely transmitted to humans through inhalation of aerosolized particles. Although self-limited febrile illness is the hallmark of acute disease, most infections remain undetected.[1,2] In children/adolescents, clinical disease is reported less frequently than in adults.[3] Lower notification rates observed in children/adolescents are commonly attributed to milder or less-specific clinical disease and subsequent underdiagnosis in younger age groups, whereas the risk of infection with *C. burnetii* is assumed to be similar in children/adolescents and adults.[4–7] Yet, cross-sectional population studies consistently report lower seroprevalence in children/adolescents than in adults, an observation that is generally attributed to increased life time risk in adults. Evidence to support any of these assumptions is scarce. Based on data from a massive single-point source Q fever outbreak in our regional public health district, causing widespread infections in a largely naive population under quasi-experimental circumstances,[1] our study is aimed to fill this gap.

MATERIALS AND METHODS

Study Area and Study Period

Our study area was equivalent to the self-reported catchment area of a large general hospital and reporting laboratory (Atrium Medical Centre Parkstad) located in the region of South Limburg, Netherlands, defined by 12 municipalities covering an area of 346 km^2 with 307,348 inhabitants. It should be noted that this is an approximation of the hospital's true catchment area and thus served only as an estimate for calculation of population-based testing and notification rates in our study. Early 2009, the area suffered a single-point source Q fever outbreak notified to the Public Health Service South Limburg (PHS) on March 24, originating from a large dairy-goat farm where 220 of 450 (48.9%) pregnant goats suffered Q fever-related abortions during that year's lambing season (February–March).[1,8] Over subsequent months, 253 residents from the study area were notified to the PHS with clinical acute Q fever. Comparing preoutbreak with postoutbreak seroprevalence in 2 regional population samples (2008 vs. 2010), we estimated that the actual number of infections was approximately 9000 and therefore much higher than the number of notified cases.[1] Incidence was highest in the municipality located closest to the outbreak farm, counting 12,703 residents at the time. Analyses largely focus on farm residents/employees/visitors and on residents from that township. Study period was marked by the time when first abortions occurred (February 2009) until culling/vaccination of goats (April 2010), when no more community subjects were diagnosed with Q fever of recent onset.

Study Populations

General Population (Residents of the Study Area)

The reporting microbiology laboratory (Atrium Medical Centre Parkstad) provided date of birth, zip code, testing dates and

screening results for all residents from the entire study area tested for Q fever on request of a general practitioner (GP) or specialty physician from February 2009 to April 2010 (n = 1228), including acute Q fever cases meeting criteria for mandatory notification (fever, pneumonia or hepatitis; seroevidence of recent infection; Table 1). No residents from outside the study area were included. Base-population demographics were from StatLine, the electronic database of Statistics Netherlands (2009). Testing and notification rates (reported cases per 100,000 of the general population) were compared in children/adolescents (0–19 years) versus adults (20 years and older) for the entire study area and also separately for the high-incidence municipality located closest to the outbreak farm.

Children/Adolescents Versus Adults With On-source (Presumed High-dose) Exposure in 2009 (Outbreak Farm Residents/Employees/Visitors)

Upon veterinary notification in March 2009, before first reports of human cases related to the outbreak, PHS strived to trace all individuals with on-premises contact to the outbreak farm since abortions started (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/C230). Given the farm's role as a proven single-point source for the entire outbreak,[1,8] on-premises contact to the outbreak farm, in terms of this study, is henceforth referred to as on-source exposure. All traceable farm residents/employees (n = 37, including mentally challenged individuals working as farm hands based on the farm's care activities) and farm visitors (n = 58) were instantly approached for seroscreening. Serological attack rates (SARs) were compared in children/adolescents (0–19 years) versus adults (20 years and older). SARs included only individuals "at risk," excluding subjects refusing blood sampling (n = 10) or showing seroprofiles indicative of past resolved Q fever [anti-*Coxiella* phase-II-IgG positive/phase-II-IgM negative (n = 9)]. Clinical cases were defined by self-reported fever/influenza-like illness and seroevidence of recent Q fever infection [phase-II-IgM and/or *C. burnetii*-polymerase chain reaction (PCR) positive].

Children/Adolescents Versus Adults With Off-source (Presumed Lower-dose) Exposure in 2009 (Residents of the Municipality Located Closest to the Outbreak Farm)

We compared seroprevalence in children/adolescents aged 0–19 years (n = 287) versus adults aged 20 years and older (n = 109) who were residents of the municipality located closest to the outbreak farm and had been shown to have no on-premises contact with the outbreak farm according to our initial contact tracing in 2009 (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/C231). Exposure meeting these criteria is henceforth referred to as off-source. A majority of children/adolescents (n = 268) were recruited through local primary and secondary schools, following consent from both parents or the guardian, and—in case of children aged ≥12 years—also from the child. Finger-stick blood samples were collected on school premises in late 2011/early 2012. Parents completed a questionnaire about children's demography, general health and history of Q fever-associated symptoms (2008–2010); outdoor exposure; and outbreak farm visits. The remaining children/adolescents (n = 19) figured among 3 convenience samples from which we also drew our comparison group of adults. The first convenience sample included a random group of consenting patients (n = 39) who had consulted the largest GP practice in the municipality from August through October 2009 for reasons unrelated to Q fever and were screened for anti-*Coxiella* phase-II and phase-I IgG and IgM. Subjects with seroevidence of acute or recent infection (anti-*Coxiella* phase-II-IgM or positive PCR if seronegative) were defined as positive. The second convenience sample included stored venipuncture sera from all municipality residents aged ≥17 years at veterinary notification who had attended the regional sexual health clinic for sexually transmitted infection testing in 2011 and the first 4 months of 2012 (n = 44). All subjects had provided consent for use of sera for scientific research. The third convenience sample included municipality residents (n = 45) who were members of a local golf club and underwent targeted seroscreening for chronic Q fever in 2013.

Laboratory Investigation

Outbreak farm residents/employees/visitors and GP patients screened in 2009 were tested for anti-*Coxiella* phase-II-IgG by enzyme-linked immunosorbent assay (ELISA, Serion ELISA classic, Institut Virion\Serion GmbH, Würzburg, Germany). Positive (>30 U/mL) and indeterminate (20–30 U/mL) samples were further tested for phase-II-IgM by Serion ELISA classic and for phase-I-IgM and phase-II-IgM and phase-II-IgG by immunofluorescence assay (IFA; Focus Diagnostics, Cypress, CA). PCR was used to exclude preseroconversion infection in seronegative subjects. Acute infections were defined by presence of anti-*Coxiella* phase-II-IgM (titer ≥ 1:32), or *C. burnetii* DNA in PCR (cycle threshold ≤36) routinely performed on all samples seronegative on initial testing.[9] Sera

TABLE 1. Q Fever Testing and Notification Rates, Study Area October 2009

Population	Total	Age 0–19 yr	Age ≥20 yr	Risk ratio	95% CI	*P*
General population study area, n (% of total)	307,348	59,404 (19.3%)	247,944 (80.7%)			
Q fever tested						
Overall, n (% of total)	1217	149 (12.1%)	1078 (87.9%)			
Testing rate, % of general population		0.3%	0.4%	0.58	0.49–0.68	<0.001
Notified cases						
Overall, n (% of total)	253	11 (4.3%)	242 (95.7%)			
Notification rate, % of tested population		7.4%	22.4%	0.33	0.18–0.58	<0.001
Attack rate, per 100 000 of general population		18.5	97.6	0.19	0.12–0.32	<0.001
General population farm's township, n (% of total)	12,703	2637 (20.8%)	10,066 (79.2%)			
Q fever tested in outbreak farm's township						
Overall, n (% of total)	360	40 (11.0%)	323 (89.0%)			
Testing rate, % of general population		1.5%	3.2%	0.47	0.34–0.65	<0.001
Notified cases in outbreak farm's township						
Overall, n (% of total)	115	3 (2.6%)	112 (97.4%)			
Notification rate, % of tested population		7.5%	34.7%	0.22	0.07–0.65	0.006
Attack rate, per 100,000 of general population		113.8	1112.7	0.10	0.03–0.32	<0.001

from school children, sexually transmitted infection clinic attendees, and golf-club members were tested for anti-*Coxiella* phase-I-IgG and phase-II-IgG by IFA, the current standard in Q fever testing. Positive samples were defined by cut-off titer ≥1:32. Laboratory tests were performed according to manufacturers' protocols.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, version 21 (IBM Inc. Somers, NY). Because the outbreak we describe was a singular event that was well defined in place and time and affected a largely naive population, we defined any serological evidence of infection with *C. burnetii* according to aforementioned criteria as incident infection, irrespective of presence of symptoms compatible with Q fever. Seroprevalence measures will, therefore, henceforth be referred to as attack rates (farm residents/employees/visitors) and cumulative incidence (individuals with off-source exposure) during the study period. Differences in notification rates (general population of the study area) and attack rates (farm residents/employees/visitors) between children/adolescents and adults were expressed as univariate risk ratios (RRs) with 95% confidence intervals (95% CI). Cumulative incidence during the study period in children/adolescents and adults with off-source exposure was compared using binary logistic regression, adjusting for sex and distance of residential address from the outbreak farm. Differences were expressed as adjusted odds ratios (OR) with 95% CIs. *P* values of <0.05 were considered statistically significant.

RESULTS

General Population

Testing and Notification Rates Were Lower in Children/Adolescents Than in Adults

Children/adolescents were compared with adults in terms of testing and notification rates over the study period, based on the general 2009 study area population (n = 307,348; Table 1). Children/adolescents represented 19% of the general population, but only 12% of subjects tested and just 4% of laboratory-confirmed notified community cases. Probability of being tested in the general population was significantly lower in children/adolescents compared with adults (risk ratio [RR]: 0.58, 95% CI: 0.49–0.68, $P < 0.001$), and so was their probability of being seropositive and notified in the group of subjects tested (RR: 0.33, 95% CI: 0.18–0.58, $P < 0.001$). Attack rate in the general population, based on notified cases, was also significantly lower in children/adolescents (RR: 0.19, 95% CI: 0.10–0.35, $P < 0.001$) than in adults. For 7 of the 11 notified children/adolescents—all symptomatic by definition—detailed self-reported questionnaire data on signs and symptoms were available: all reported flu-like illness/fever, 6 reported headaches, 4 serious fatigue and 1 reported cough. Comparing children/adolescents and adults resident in the municipality located closest to the outbreak farm, differences in testing rate (RR: 0.47, 95% CI: 0.34–0.65, $P < 0.001$), notification rate (RR: 0.22, 95% CI: 0.07–0.65, $P = 0.006$) and attack rate (RR: 0.10, 95% CI: 0.03–0.32, $P \leq 0.001$) were even more pronounced.

Children/Adolescents Versus Adults With On-source (Presumed High-dose) Exposure: Attack Rates in Children/Adolescents Were Equally High as in Adults

We compared serological and clinical attack rates (CARs) in children/adolescents with the rates in adults in at-risk individuals (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/C230). Among traced outbreak farm residents/employees, 11 of 37 (30%) were aged <20 years. SAR of acute infection was high: 100% in children/adolescents versus 89% in adults. SAR in traced farm visitors [11 of 58 (19%) children/adolescents], where exposure intensity was lower, was 44% in children/adolescents versus 59% in adults. CAR, based on a small subgroup of seropositives for whom we had questionnaire data on clinical symptoms, was 100% (6 of 6) in children/adolescents versus 83% (24 of 29) in adults. None of the differences in SAR or CAR between age groups were statistically significant.

Children/Adolescents Versus Adults With Off-source (Presumed Lower-dose) Exposure: Cumulative Incidence of Infection and Notification Rates in Children/Adolescents Were Lower Than in Adults

Comparing cumulative incidence of *C. burnetii* infection during the study period in a sample of children/adolescents with that in adults from the municipality that had suffered the bulk of notified clinical cases in the study area and had the highest incidence of infections in 2009, we show that incidence distribution followed a reversed u-shape pattern across 20-year age groups (Fig. 1, see Table, Supplemental Digital Content 2, http://links.lww.com/INF/C231 and Table, Supplemental Digital Content 3, http://links.lww.com/INF/C232). Estimated cumulative incidence of infection in children/adolescents aged 0–19 years compared with that in the overall population of adults aged 20 years and above was 4.5% (13 of 287) versus 11.0% [12 of 109; adjusted OR: 0.36 (95% CI: 0.16–0.84, $P = 0.02$)] during the study period. Difference was most pronounced in children/adolescents aged 0–19 years

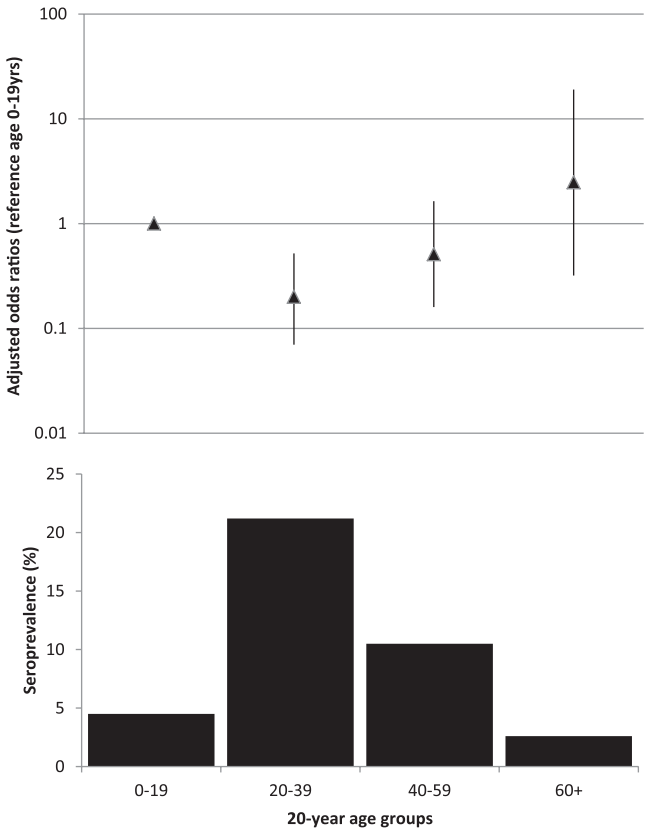

FIGURE 1. Cumulative incidence of *Coxiella burnetii* infection in community residents with off-source exposure by 20-year age groups (below) and corresponding adjusted OR (age 0–19 years vs. other age groups) with 95% CI (above, logarithmic scale).

versus adults aged 20–39 years (OR: 0.18, 95% CI: 0.06–0.49, $P = 0.001$). Cumulative incidence was also lower in children/adolescents compared with adults aged 40–59 years (OR: 0.38, 95% CI: 0.11–1.29, $P = 0.12$) and slightly higher than in adults aged ≥60 years (OR: 1.60, 95% CI: 0.20–12.74, $P = 0.66$), but these differences were less outspoken and not statistically significant. We also compared 10-year age groups, using children aged 0–9 years as reference. This did not alter the trend or statistical significance of our findings based on 20-year age categories: the difference between children (0–9 years) and adolescents (10–19 years) was statistically not significant, and neither was the difference between adults aged 20–29 and 30–39 years, nor the difference between adults aged 40–49 and 50–59 years, whereas cumulative incidence in children aged 0–9 and 10–19 years differed significantly from adults aged 20–29 and 30–39 years (data not shown). Although none of the children/adolescents and adults included belonged to the group of individuals with on-premises farm contacts identified through initial contact tracing, we excluded, by way of sensitivity analysis, 10 children with a questionnaire-reported outbreak farm visit in 2009 (all seronegative) from our analyses. No similar data were available for the included adults. Sensitivity analysis did not affect the magnitude or statistical significance of our findings. Projecting observed cumulative incidences to the municipality's general population (2637 children/adolescents vs. 10,066 adults), we estimated that 119 infections occurred in children/adolescents versus 1108 in adults. Given that only 3 cases were notified in children/adolescents in 2009, versus 108 in adults, the notification rate was just 2.5% (3 of 119) in children/adolescents versus 10.4% (108 of 1108) in adults (RR: 0.26, 95% CI: 0.08–0.80, $P = 0.02$). None of the seropositive children from this population had questionnaire-reported episodes of disease, health problems or deterioration of health in 2008–2010.

DISCUSSION

To our knowledge, our study is first to compare the risk of *C. burnetii* infection in a large group of children/adolescents and adults in an outbreak setting that was distinguished by the presence of a proven single-point source affecting a largely naive population and was well defined in time and place, allowing us to estimate true cumulative incidence of infection in both groups. Summarizing, we found that, not unexpectedly, notified clinical Q fever was less common in infected children/adolescents than in adults. Contrary to common belief, however, children/adolescents were less likely to become infected than adults in the first place, at least after community (off-source) exposure to *C. burnetii*, contributing significantly to infrequent Q fever reporting in children/adolescents. On-source exposure, by contrast, with higher presumed dosage, was associated with high serological and CARs in children/adolescents similar to those in adults. Our findings may offer a useful tool for enhanced public health risk assessment in Q fever outbreaks, allowing communicable disease professionals to estimate—in terms of *C. burnetii* infections—the true extent of an outbreak in different age groups and under varying exposure conditions. In addition, our findings may assist clinicians in their decision making, as children with on-source or near-source exposure, particularly those with underlying risk factors, may require a more proactive approach in terms of testing and treating, even in the absence of any signs or symptoms than children with community exposure. The possible protective role of young age in the pathophysiology of Q fever should be further explored in animal models and prospective studies. Evidence from our study may also provide for better understanding of divergent findings from previous outbreak and cross-sectional population seroprevalence studies, as we show below.

Literature comparing incidence of *C. burnetii* infection in children/adolescents with that in adults is scarce. Population seroprevalence studies almost invariably show seropositive rates to be significantly lower in children/adolescents than in adults.[10–25] Although these findings are essentially cross-sectional in nature and may reflect cohort effects or life time risk of exposure increasing with age, they can—according to our findings—also be explained by lower infection risk in children/adolescents under off-source exposure conditions. Interestingly, an active surveillance study among villagers from Cyprus, Greece, found both seroprevalence and incidence to be lower in children than in adults, and to our knowledge it is the only, albeit smaller, study—along with our own—to investigate age-specific incidence and report lower risk of infection with *C. burnetii* in children/adolescents compared with adults.[26] Several studies support the notion that Q fever may be a common problem in African children, and that differences between children/adolescents and adults in Africa, if any, may be less pronounced than in our study.[27–30]

By contrast, outbreak studies where exposure took place close to a known source in confined indoor settings (such as a household or a school building) tend to show that children/adolescents under such conditions have the same high risk of infection as adults.[31–35] These findings may be explained by the high dosage of infectious agent incurred under these conditions, supported by observations from our study, where SARs in children/adolescents with on-source exposure to the outbreak farm were equally high as in adults. The same holds true for occupational exposure studies investigating human seroprevalence on farms, where exposure—though not outbreak related—most likely took place on-source, that is, on the farm premises, possibly over prolonged periods of time.[36–39] In these studies, seroprevalence in children/adolescents tended to be slightly but, in general, not significantly lower than the seroprevalence in adults.

Evidence regarding CARs of Q fever in children/adolescents versus adults is limited to a small number of outbreak studies, with divergent findings.[40–44] In general, outbreak studies characterized by outdoor exposure, usually affecting a wider geographical area, report CARs in children/adolescents to be lower than in adults. This is consistent with lower notification rates in children/adolescents in the general population that we found in our study, and with the fact that seropositive children/adolescents in our group of individuals with off-source exposure had no parent-reported symptoms, suggesting that clinical Q fever in children/adolescents was indeed a rare event in the community. Interestingly, studies describing outbreaks with on-source exposure in a confined indoor setting consistently report not just high SARs, but also high CARs in children/adolescents, similar to those in adults.[31–35] Again, these observations may be explained by high dosage overriding any protective effects related to young age, as supported by evidence from our study in individuals with on-source exposure.

Lower risk of *C. burnetii* infection in children/adolescents that we found in our study may be related to unidentified age-specific (eg, behavioral) exposure differences or, alternatively, to biological protective effects conferred by young age. Although we have no data to assess the mechanisms underlying lower risk in children/adolescents, we think this finding deserves further exploration, as it may advance our understanding of Q fever epidemiology and pathophysiology, opening prospects for better prevention and treatment. The low seroprevalence we found in subjects ≥60 years may have an immunological basis. A recent study showed that humoral and cellular immune response in elderly subjects with risk factors for chronic Q fever was relatively modest after Q fever vaccination.[45] Immunosenescence has been observed in other vaccination studies.[46,47] In our study, it may have been accentuated by lack of boostering after primary exposure in 2009, as regional zoonotic transmission was limited to that year.

Our study has limitations. First, a majority of individuals included in our study were screened with varying delays after the outbreak, potentially introducing time-related bias. It should be noted that regional seroprevalence in the general population in 2008, the year before the outbreak, was as low as 0.5%, arguing against any large-scale preoutbreak transmission.[1] In addition, culling of pregnant goats by the end of 2009 and vaccination of the remaining goat population effectively curbed zoonotic transmission, as confirmed by the fact that in spite of continued GP-requested testing for Q fever, no new cases with recent disease onset were reported to the local PHS beyond 2009, marking effectively the end of the outbreak. We thus feel confident that age cohort and life time risk effects did not apply to our study or confound our findings. Although decreasing sensitivity of the assay employed in screening our subjects, because of waning of antibodies over the years, may be an issue, several authors have suggested long-term persistence of anti-*Coxiella* IgG, with IFA findings remaining positive for periods of 10 years or longer.[48–51] Seroprevalence in adults screened in our study seemed to be remarkably stable, irrespective of the year of screening. Although similar data on children are scarce, we found that at least 1 girl from our cohort of schoolchildren was phase-II-IgG positive in IFA in 2009 (aged 3 years) and persistently IFA positive in 2012. Recall bias may have affected parents' recall of symptomatic episodes in our group of children. However, more serious symptoms, like those requiring absence from school, GP visits or treatment, would unlikely have gone unreported. Second, for lack of detailed individual exposure histories, we used mean distance of residential address from the outbreak farm as a well-established proxy of exposure intensity in children/adolescents and adults.[1] Even though mean distance did not differ between children/adolescents and adults, distance was included as a covariate in our statistical comparisons but did not affect outcomes. Moreover, it should be noted that during the 2009 outbreak, schools did not implement interventions to minimize infection risk, for example, by keeping children/adolescents indoors during breaks, which otherwise might have explained lower incidence of infection in children/adolescents. In addition, it is generally assumed that total outdoor exposure times in children/adolescents are higher than in adults, suggesting they should actually have a higher exposure risk than adults.[52] Other factors which we, for lack of data, were unable to adjust for may have acted as confounders and need further exploration. For example, a recent study showed that current cigarette smoking was nearly as strong a risk factor as living in the immediate vicinity of an affected dairy-goat farm.[53] Third, self-selection bias related to a history of pertinent symptoms or high-risk exposures may have influenced individual subjects' decision whether or not to participate, allowing for bias in both directions.

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