

## *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands

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### SUMMARY

In this study, *Coxiella burnetii* seroprevalence was assessed for dairy and non-dairy sheep farm residents in The Netherlands for 2009–2010. Risk factors for seropositivity were identified for non-dairy sheep farm residents. Participants completed farm-based and individual questionnaires. In addition, participants were tested for IgG and IgM *C. burnetii* antibodies using immunofluorescent assay. Risk factors were identified by univariate, multivariate logistic regression, and multivariate multilevel analyses. In dairy and non-dairy sheep farm residents, seroprevalence was 66·7% and 51·3%, respectively. Significant risk factors were cattle contact, high goat density near the farm, sheep supplied from two provinces, high frequency of refreshing stable bedding, farm started before 1990 and presence of the Blessumer breed. Most risk factors indicate current or past goat and cattle exposure, with limited factors involving sheep. Subtyping human, cattle, goat, and sheep *C. burnetii* strains might elucidate their role in the infection risk of sheep farm residents.

**Key words:** Coxiellae, Q fever, risk assessment, serology, zoonoses.

### INTRODUCTION

Q fever, caused by *Coxiella burnetii*, is a worldwide zoonosis with goats, sheep, and cattle as primary sources for human infections [1]. Humans are usually infected by inhalation of contaminated aerosols originating from parturient animals and their birth

products [1–3]. Acute Q fever presents itself as a self-limiting febrile illness, pneumonia or hepatitis, with a small proportion developing chronic infections (mainly endocarditis and vascular infections) [4, 5].

From 2007 until 2009, large Q fever outbreaks occurred in The Netherlands, with over 3500 human cases notified [6]. Abortion waves at dairy goat farms were the primary source of these infections [7–9]. Between 2006 and 2008, *C. burnetii* abortion waves occurred on two dairy sheep farms [9]. Infected non-dairy sheep farms were not associated with an increased number of human cases living near these farms [10], although cases occurred in

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individuals living a small distance from or having direct contact with non-dairy sheep in The Netherlands [11, 12]. Internationally, several sheep-related Q fever outbreaks have been reported [13–19].

In The Netherlands, sheep farms can be distinguished from dairy farms and fat lamb-producing farms. There is a small dairy sheep industry with <50 farms, in which sheep are usually milked twice a day during several months each year. The number of sheep per farm differs from <50 to almost 1000 with most kept outdoors for part of the year. On the fat lamb-producing sheep farms the sheep are kept outside, except for a few weeks around lambing, which usually occurs inside. Except for meat production, non-dairy sheep are also kept for breeding purposes or nature management.

So far, no international studies have addressed the seroprevalence and risk factors for acquisition of *C. burnetii* infection in sheep farmers and their household members. Therefore, our aim was to determine the *C. burnetii* seroprevalence in both dairy and non-dairy sheep farmers and their household members, and for the large non-dairy sector, to identify individual and farm-related risk factors for seropositivity.

## MATERIAL AND METHODS

All dairy sheep and non-dairy sheep farms in The Netherlands with at least 100 breeding ewes in November 2008, according to the national identification and registration database, were eligible. A minimum of 100 ewes, considered to be a professional farm, was chosen because in the early stage of the Dutch epidemic it was clear that only (relatively large) commercial (dairy goat) farms were incriminated as a potential source; no obvious role for small farms was observed [9]. Besides, smaller hobby farms have different management and farm residents of those farms are assumed to have a more limited exposure to sheep-related pathogens compared to commercial farms. Between September and December 2009, 32 dairy sheep farmers were approached for the study. In addition, in March and April 2010, 1344 non-dairy sheep farmers were approached for participation. At the time of inclusion in 2010, those farms with at least 60 unvaccinated breeding animals were kept in the study. Farms with vaccinated sheep were excluded because in this integrated human-veterinary study the sheep at these farms were likely to be seropositive due to

vaccination; vaccine-induced and naturally induced seroresponses cannot be distinguished to assess the true seroprevalence from natural infection. Second, we assumed that the infection rate for farm residents could be different for farms with vaccinated sheep (leading to reduced exposure) compared to farms with unvaccinated sheep. About 3 weeks after the initial invitation, all non-responding farmers were sent a written reminder. Because of the small number, dairy sheep farmers who did not respond to this second invitation were contacted by telephone.

After written informed consent, a maximum of three persons were selected from each farm, i.e. the farmer and a maximum of two family members aged  $\geq 12$  years residing at the farm; in some instances other persons working or living on the farm were selected. Each participant received a questionnaire addressing individual-based risk factors like age, gender, profession, ownership or contact with ruminants and pets, consumption of unpasteurized milk, medical history, and contact with agricultural products. In addition, the farm owner or farm manager completed a farm-based questionnaire addressing characteristics like farm hygiene and management, herd size, presence of other livestock and pets, stable environment, and lambing season characteristics. Separate farm-based questionnaires were developed for dairy farms and non-dairy farms because of clear differences in farm management. A professional laboratory assistant visited the farms to collect blood samples from all participating individuals for serology. All data of the dairy sheep farms were collected between September 2009 and September 2010, for the non-dairy sheep farms data were collected between April and September 2010. The Medical Ethical Commission of the University Medical Center Utrecht approved the study protocol (no. 09–189/K).

### Serological analysis

Serum samples were tested for *C. burnetii* IgM and IgG antibodies, both phases I and II, using an indirect immunofluorescence assay (IFA) with a screening dilution of 1:32. Participants without any positive antibody result and participants with a solitary IgM phase I or solitary IgM phase II result were classified as seronegative. All other outcomes were classified as seropositive. Those with IgM phase II antibodies were designated as ‘relatively recent infections’ and included possible current infections. The term ‘relatively recent’ was chosen as IgM phase II is found

to persist in the majority of cases for 1 year post-infection and may even persist up to 4 years post-infection [20, 21] (C. C. H. Wielders, personal communication). Seropositives without IgM phase II antibodies were designated as 'past infections'. As the latter group also includes possible chronic infections, a further distinction was made between serological profiles that had IgG phase I  $\geq 1:1024$  indicative for a chronic infection according to the new Dutch consensus guidelines [22].

## Statistical analyses

### *Dairy sheep farms*

All data were analysed with SAS, version 9.2 (SAS Institute Inc., USA). For the dairy sheep farms in The Netherlands, participation bias was investigated by comparing participating and non-participating farms with regard to herd size, urbanization degree and region. The seroprevalence of *C. burnetii* in residents and the corresponding 95% confidence interval (CI) were calculated. Descriptive statistics were performed by analysing frequency tables and studying distributions of continuous variables. No risk factor analysis was performed because of the small number of participants.

### *Non-dairy sheep farms*

To study participation bias, participating and non-participating farms were compared with regard to herd size, cattle, sheep, and goat density in the surroundings, urbanization degree, region, situated inside or outside a compulsory Q fever vaccination area, number of bulk-milk-positive dairy goat or dairy sheep farms in a radius of 5 and 10 km, and distance in metres to the closest bulk-milk-positive small ruminant farm.

The seroprevalence of *C. burnetii* and the corresponding 95% CI were calculated. For descriptive statistics, frequency tables were analysed. In addition, distributions of continuous variables were studied, and if not linearly related to the outcome variable, continuous variables were recoded into classes.

Univariate logistic regression analysis was performed to assess the main factors associated with *C. burnetii* seropositivity at the individual level [ $P < 0.20$  in the likelihood ratio test ( $-2LL$ )]. Variables with  $< 20$  participants in one risk category were excluded. Age was always kept in the model because of the frequent association with Q fever

seropositivity in the literature. Proxy outcomes, such as sheep seropositivity, were not included in the multivariate analysis. If several variables, which were associated in the univariate analysis, were interrelated, a preferred variable was chosen and related variables were excluded. The preferred variable was chosen based on the most informative value, the strongest association or most relevant exposure (exposure at own farm instead of comparable exposure at other farms). All identified individual variables were analysed with a manual backwards elimination procedure until all variables were significant at the 10% significance level in the likelihood ratio test, starting with a full multivariate logistic regression model.

Subsequently, potential risk factors derived from the farm-based questionnaire were analysed by univariate multilevel analyses considering clustered farm-based data for all persons within the same farm, using a unique farm number as cluster variable. All farm variables which were significant in the univariate analysis ( $P < 0.20$ ), were analysed with a manual backward elimination procedure starting with a full multilevel model.

Finally, both the individual and farm-based characteristics from the two final submodels were combined in a multivariate multilevel analysis to identify the independent risk determinants for *C. burnetii* seropositivity. The final model fit was assessed by the quasi-likelihood under the independence model criterion (QIC) goodness-of-fit statistic for generalized estimation equation (GEE) models.

## RESULTS

### *Dairy sheep farms*

Out of the 32 invited farms, 12 participated (response rate 37.5%). The participating farms were all situated in a rural area ( $< 500$  addresses/km<sup>2</sup>). Participating and non-participating farms were comparable with regard to urbanization degree and province distribution. However, participating farms had a median number of 529 sheep (range 143–1163) vs. the significantly lower median of 353 sheep (range 96–730) for non-participating farms ( $P = 0.03$ ).

Twenty-seven study participants (mean age 38.7 years, range 14–61, 63% male), provided a blood sample. Overall, 18 (66.7%) participants were seropositive: 80.0% for the 15 farmers (12 males), and 50.0% for the 12 household members (five children, five female spouses, one male spouse, one

seasonal worker). Three (11.1%) participants had a relatively recent *C. burnetii* infection (IgM phase II antibodies). None consulted their general practitioner or were hospitalized because of influenza-like illness or fever. One participant had an IgG phase I titre of  $\geq 1:1024$ , indicating a possible chronic case [22].

### Non-dairy sheep farms

#### *Non-response analyses*

Out of the 1344 approached farms, at least 32 appeared to be no longer eligible because they had <60 animals at inclusion or had vaccinated all their sheep. Of the remaining 1312 farms, 119 participated in the study (response rate 9.1%).

A significant difference was found for sheep density in the 5-km radius of participating and non-participating farms, 34.5 (range 1.8–143.6) and 47.5 (range 1.0–162.9) sheep/km<sup>2</sup> in the 5-km radius (excluding own sheep), respectively ( $P=0.01$ ). In addition, the number of sheep was borderline significantly higher at the participating farms (median 191 sheep, range 102–1310), compared to the non-participating farms (median 167 sheep, range 100–2857). For the other variables, no significant differences were found between participating and non-participating farms (Table 1).

#### *Descriptive characteristics*

The 119 participating farms were mainly situated in the provinces of Noord-Holland and Friesland, commonly (90.8%) situated in rural areas (<500 addresses/km<sup>2</sup>) and the most common breeds at the farms were Texel (57.0%) and Swifter (46.5%). The farms were mainly started after 1950 (9.6% 1875–1950, 39.4% 1951–1980, 51.0% after 1980). Out of the 114 farms with a farm-based questionnaire, 23 (20.2%) kept one or more goats, 45 (39.5%) kept dairy cattle and/or beef cattle, and 13 (11.4%) other farms reported that cattle were present on their pastures. The farms could have one or more function; 95 (83.3%) farms kept sheep for meat production, 53 (46.5%) farms for rearing, and 20 (17.5%) farms for nature management. Of those 20 farms, 12 farms kept their sheep exclusively for nature management.

From the 119 farms, 271 persons provided a blood sample (mean age 47, range 12–93 years, 55% male). Of those, 266 completed the individual self-administered questionnaire and from 261 individuals

information was available from the farm-based questionnaire.

*C. burnetii* seroprevalence was 51.3% (95% CI 45.5–57.4). In the univariate analysis, seroprevalence was significantly higher for farmers (58.8% vs. 36.3% for spouses) and for males (57.7% vs. 43.4% for females). Out of the 271 participants, seven (2.6%) had a relatively recent infection (IgM phase II antibodies). No participant had an IgG phase I titre suggestive for chronic infection.

Although the seroprevalence of the farm residents was higher for those living on a dairy sheep farm, the difference was not statistically significant [odds ratio (OR) 1.9, 95% CI 0.8–4.4] for dairy sheep farmers vs. non-dairy sheep farmers).

#### *Univariate analyses at individual and farm level*

All individual and farm-based variables, which were tested in the univariate analysis for relationship with human *C. burnetii* seropositivity, are displayed in Tables 2 and 3.

#### *Multivariate and multilevel analyses*

In the multivariate analyses, from 23 individual variables which were associated in the univariate analysis, four were independently associated with *C. burnetii* seropositivity (Table 4). In addition, 10/23 farm-based variables included in the multilevel analyses were significantly independent risk or protective factors and together were used as the full multilevel start model (Table 5).

#### *Combined multilevel analyses of individual and farm-based factors*

In the final combined multilevel model, significant risk factors were contact with cattle at own or other farm, past employment in the cattle sector, high goat density in the vicinity of the farm, living or working at a farm that was started in 1990 or later, the presence of Blessumer breed on the farm, cattle on the same pastures used by sheep, although not simultaneously with the sheep, high frequency of refreshing the bedding in the sheep stables, and sheep supplied from the provinces of Groningen or Noord-Holland (Table 6). Borderline significant risk factors were age 40–49 years, and presence of dairy cattle during the stabling period of the sheep. In addition, sheep lambing outside was a significant protective factor, and air entering the stable through the door was a borderline significant protective factor.

Table 1. Non-response analyses of non-dairy sheep farms, comparison of participating and non-participating farms

Numerical variables	Participating farms (N=119) Median	Non-participating farms (N=1193) Median	P value
Number of sheep	191	167	0.05
Cattle density (number of cattle/km <sup>2</sup> in the municipality)*	134.7	135.5	0.16
Cattle density without veal calves (number of cattle/km <sup>2</sup> in the municipality)*	114.7	119.5	0.10
Goat density (number of goats/km <sup>2</sup> excluding own animals in a 5-km radius)*	2.6	3.5	0.17
Sheep density (number of sheep/km <sup>2</sup> excluding own animals in a 5-km radius)*	34.5	47.5	0.01
Closest Q fever bulk-milk-positive dairy goat or dairy sheep farm (metres)*	13960	13806	0.70
Number Q fever bulk-milk-positive dairy goat or dairy sheep farms in a 5-km radius*	0 (min=0, max=2)	0 (min=0, max=4)	0.62
Number Q fever bulk-milk-positive dairy goat or dairy sheep farms in a 10-km radius*	0 (min=0, max=4)	0 (min=0, max=9)	0.71
<b>Categorical variables</b>	<i>n</i> (%)	<i>n</i> (%)	<i>P</i> value
Inside vaccination area	20 (16.8)	181 (15.2)	0.64
Outside vaccination area	99 (83.2)	1012 (84.8)	
Urbanization			
Very high urban area*†	0 (0.0)	2 (0.2)	0.37
High urban area	0 (0.0)	3 (0.3)	
Moderate urban area	4 (3.3)	14 (1.2)	
Minor urban area	7 (5.9)	84 (7.0)	
Rural area	108 (90.8)	1086 (91.3)	
Province			
Drenthe*	4 (3.4)	57 (4.8)	0.52
Flevoland	1 (0.8)	9 (0.8)	
Friesland	18 (15.1)	213 (17.9)	
Gelderland	14 (11.8)	170 (14.3)	
Groningen	11 (9.2)	93 (7.8)	
Limburg	4 (3.4)	23 (1.9)	
Noord-Brabant	12 (10.1)	74 (6.2)	
Noord-Holland	29 (24.4)	241 (20.3)	
Overijssel	11 (9.2)	86 (7.2)	
Utrecht	2 (1.7)	48 (4.1)	
Zeeland	2 (1.7)	49 (4.1)	
Zuid-Holland	11 (9.2)	126 (10.6)	

N, Total number of individuals.

\* Four missing values at non-participating farms.

† Urbanization degree: very high urban area >2500 addresses/km<sup>2</sup>; high urban area = 1500–2500 addresses/km<sup>2</sup>; moderate urban area = 1000–1500 addresses/km<sup>2</sup>; minor urban area = 500–1000 addresses/km<sup>2</sup>; rural area <500 addresses/km<sup>2</sup>.

## DISCUSSION

### Seroprevalence

The seroprevalence of non-dairy (51.3%) and dairy sheep farm residents (66.7%) is clearly higher compared to the seroprevalence estimate of 2.4% in the general population before the outbreak occurred in The Netherlands in 2006–2007. It is even higher compared to the seroprevalence found in a small

community in the epicentre of the Q fever outbreak in 2007 (25.1%), and in blood donors in the most Q fever-affected areas in 2009 (12.2%), indicating that sheep farm residents have an increased life-time risk of acquiring a *C. burnetii* infection compared to the general Dutch population [7, 23, 24].

The observed seroprevalence in Dutch sheep farm households is also high compared to a study of sheep farmers in Sweden (28.5%) [25], and of

Table 2. *Univariate logistic model of individual factors related to C. burnetii seropositivity in non-dairy sheep farm residents (P < 0.20, -2LL)*

Variable	Category	Frequency (N) (N = 266)	Sero- prevalence (%)	OR (95% CI)
Gender*	Male	144	57.6	1.77 (1.09–2.88)
	Female	122	43.4	Reference
Age (years)*	12–19	21	57.1	2.04 (0.72–5.76)
	20–39	45	51.1	1.60 (0.70–3.63)
	40–49	68	58.8	2.18 (1.03–4.63)
	50–59	84	50.0	1.53 (0.74–3.13)
	>60	48	39.6	Reference
Work and/or live on farm	Work and live	188	53.7	1.61 (0.83–3.15)
	Work, but not live	35	48.6	1.31 (0.53–3.22)
	Not work, but live	43	41.9	Reference
Function	Farmer	136	58.8	2.51 (1.42–4.44)
	Spouse	80	36.3	Reference
	Child†	39	53.9	2.05 (0.94–4.46)
	Other‡	11	54.6	2.11 (0.59–7.53)
How often in stable	Every day	185	55.7	Reference
	Every week	56	41.1	0.56 (0.30–1.02)
	Every month	10	50.0	0.80 (0.22–2.84)
	Less than once a month/never	15	33.3	0.40 (0.13–1.21)
Amount of work at farm*	Full working week	61	63.9	2.39 (1.25–4.56)
	Up to half a working week	97	52.9	1.49 (0.86–2.59)
	Never/occasionally	108	42.6	Reference
Feed sheep*	Yes	225	55.6	3.41 (1.63–7.14)
	No	41	26.8	Reference
Load and unload sheep	Yes	194	56.2	2.14 (1.23–3.72)
	No	72	37.5	Reference
General healthcare of sheep	Yes	201	55.7	2.15 (1.21–3.82)
	No	65	36.9	Reference
Remove manure	Yes	180	57.8	2.31 (1.36–3.92)
	No	86	37.2	Reference
Spread manure*	Yes	124	58.9	1.80 (1.10–2.92)
	No	142	44.4	Reference
Clean stables	Yes	167	56.3	1.75 (1.06–2.89)
	No	99	42.4	Reference
Administrative work	Yes	193	54.4	1.62 (0.94–2.78)
	No	73	42.5	Reference
Wear overalls or boots*	Yes	234	54.3	3.03 (1.35–6.84)
	No	32	28.1	Reference
Contact with cattle at own or other farm*§	Yes	172	63.4	4.29 (2.49–7.40)
	No	94	28.7	Reference
Contact with horses at own or other farm*§	Yes	145	59.3	2.07 (1.27–3.38)
	No	121	41.3	Reference
Contact with pigs at own farm*§	Yes	24	37.5	0.54 (0.23–1.29)
	No	242	52.5	Reference
Indirect contact with poultry at own farm*	Yes	93	57.0	1.44 (0.87–2.39)
	No	173	48.0	Reference
Indirect contact with rats at own farm*	Yes	45	64.4	1.93 (0.99–3.76)
	No	221	48.4	Reference
Contact with goats at other farm*§	Yes	32	62.5	1.70 (0.79–3.63)
	No	234	49.6	Reference
Contact with sheep at other farm*§	Yes	102	60.8	1.89 (1.14–3.12)
	No	164	45.1	Reference
Contact with dogs at other farm*§	Yes	112	58.9	1.72 (1.05–2.82)
	No	154	45.5	Reference

Table 2 (cont.)

Variable	Category	Frequency ( <i>N</i> ) ( <i>N</i> = 266)	Sero- prevalence (%)	OR (95% CI)
Indirect contact with poultry at other farm	Yes	38	63.2	1.78 (0.87–3.61)
	No	228	49.1	Reference
Indirect contact with cats at other farm*	Yes	81	59.3	1.60 (0.95–2.72)
	No	185	47.6	Reference
Direct contact with wool*	Yes	113	60.2	1.89 (1.15–3.09)
	No	153	44.4	Reference
Direct contact with hay, straw or animal feed*	Yes	228	54.8	2.98 (1.41–6.29)
	No	38	29.0	Reference
Direct contact with raw milk	Yes	72	62.5	1.91 (1.10–3.32)
	No	193	46.6	Reference
Drink raw milk from cattle*	Yes	45	66.7	2.17 (1.11–4.26)
	No	221	48.0	Reference
Direct contact with cattle manure	Yes	110	68.2	3.30 (1.97–5.52)
	No	155	39.4	Reference
Direct contact with live-born animals during lambing period	Yes	246	53.3	3.42 (1.21–9.69)
	No	20	25.0	Reference
Direct contact with dead-born animals/placenta*	Yes	210	54.3	1.84 (1.01–3.35)
	No	56	39.3	Reference
Tick bite*	Yes	61	42.6	0.64 (0.36–1.14)
	No	205	53.7	Reference
Did not work in animal husbandry/agriculture in the past	Yes	114	39.5	0.44 (0.27–0.72)
	No	152	59.9	Reference
Employment in cattle sector in the past*	Yes	107	64.5	2.49 (1.50–4.14)
	No	159	42.1	Reference
Worked in animal transport/ transport of agricultural products in the past*	Yes	37	70.3	2.56 (1.21–5.42)
	No	229	48.0	Reference
As a child lived at:	Cattle farm	151	59.6	2.04 (1.18–3.53)
	Other kind of farm	34	35.3	0.75 (0.33–1.73)
	No farm	81	42.0	Reference
As a child worked in animal care/with manure/hay/in vegetation care*	Yes	178	56.2	1.85 (1.10–3.11)
	No	88	40.9	Reference

*N*, Total number of individuals; OR, odds ratio; CI, confidence interval, –2LL, likelihood ratio test.

\* Variables included in subsequent multivariate individual analyses before manual backward elimination.

† Children aged <18 years (*n* = 17) and older children (*n* = 22) of the farmer.

‡ Employees, shepherds, other family members.

§ See animals at <5 m or touch animals.

|| See animals at <5 m.

farmers from all types of farms: 17.8% in Poland, and 27.3% in the UK [26, 27]. Generally, it is difficult to compare international seroprevalence studies, because most studies use different tests or cut-off values. The cut-off value of the test in our study ( $\geq 1:32$ ) was chosen because it allowed comparison with other population surveys conducted in The Netherlands [23, 28].

Dairy sheep farm residents had a higher seroprevalence compared to non-dairy sheep farm residents. Although no statistically significant difference in seroprevalence was found between the residents of both

farm types, this might be due to lack of power because of the small number of participants from dairy sheep farms. In this study it was impossible to assess which risk factors were responsible for the higher seroprevalence in dairy sheep farm residents, due to the low number of participating dairy sheep farm residents. In addition, because of the differences in farm management, the farm-based questionnaires of both farm types were not the same, therefore pooling the analysis with the other sheep farm residents to increase power was not an option. Specific research, targeting all current dairy sheep farms

in The Netherlands ( $n \sim 40$ ), might elucidate further risk factors next to the higher sheep seroprevalence, explaining the higher seroprevalence in dairy sheep farm residents. Nevertheless, it might well be that dairy farm residents were more exposed to *Coxiella*, as the seroprevalence in dairy sheep at these same farms was significantly higher compared to that of non-dairy sheep (data not shown). A higher vulnerability for infection of breeds selected for milk production rather than for disease resistance has previously been observed for dairy cattle, dairy sheep, and dairy goats [29, 30]. In addition, dairy sheep are more often housed in stables compared to non-dairy sheep which spend most of the year outside. Indoor housing might facilitate the spread of *C. burnetii* in dairy sheep and to humans. Moreover, the higher seroprevalence in dairy farm residents might be explained by more intense contact with dairy sheep.

The seroprevalence of the dairy sheep farm residents (66.7%) was comparable to the seroprevalence of dairy goat farm residents (68.7%) in The Netherlands [28]. Furthermore, the percentage of relatively recent infections (clinical status unknown as no questions addressed current Q fever compatible symptoms) in the dairy sheep farm residents (11.1%) is comparable to that of the dairy goat farm residents (11.2%) [28]. Additionally, the percentage of participants with an indication for a possible chronic infection is also similarly high for dairy sheep and dairy goat farm residents (3.7% and 4.1%, respectively) [28]. In contrast, the percentage of relatively recent infections and possible chronic infections are lower for non-dairy sheep farm residents (2.6% and 0%, respectively). Therefore, currently *C. burnetii* infection seems to be a more serious and on-going health problem in dairy goat and dairy sheep farm residents compared to non-dairy sheep farm residents, although the numbers are relatively small.

Although numbers are too low to draw any conclusion and do not allow for valid statistical testing, the 10 (three from dairy and seven from non-dairy farms) relatively recent (IgM phase II positive) cases were generally younger (median 37 years *vs.* median 50 years for the seronegatives), were more often male (80% *vs.* 48%) and more often lived on a dairy sheep farm (30% of the recently infected *vs.* 6% of the seronegatives). This may point to ongoing infections especially in male dairy sheep farm residents, in the relatively early days of their contact with sheep.

### Risk and protective factors for non-dairy sheep farm residents

One of the protective factors for *C. burnetii* seropositivity was sheep lambing outside. Farm residents might be less exposed to contaminated aerosols in that situation, compared to lambing inside stables.

In addition, several risk factors for *C. burnetii* seropositivity were identified in this study. McCaughey *et al.* [31] suggested in his study in the general population (age 12–64 years) that most people acquired *C. burnetii* infection between ages 25 and 34 years and after that age seroprevalence remained stable. This age trend was not seen in our study; sheep farm residents had already a high seroprevalence at young age (12–19 years). This might be explained by exposure to infected animals at a young age. The highest seroprevalence found in humans (age 40–49 years), matches the most common age group of notified clinical Q fever cases in The Netherlands [9]. The increased risk at this age seems not to be explained by differences in specific work activities, frequency of cattle contact, or hours worked. Perhaps host factors play a role in the increased risk, or it generally reflects regular exposure to the bacterium and repeated development of antibodies (booster effect), not adequately measured by the questions in the questionnaire.

Animal movement is a known risk factor for the transfer of microorganisms and should be discouraged [32, 33]. Why specifically supply of sheep from the northern provinces of Noord-Holland and Groningen showed an independent increased risk for infection of the farm residents is not clear. The seroprevalence in sheep in these two provinces was not significantly different from prevalences in other provinces, both in the current study (B. Schimmer *et al.*, unpublished data) and in a previous study in 2008 using convenience serum samples from sheep [30].

It is also unknown why the fact that a farm started before 1990 was a risk factor. No change in farm management is known around that year that could influence the risk of a *C. burnetii* infection.

Having the Blessumer sheep breed on the farm was the next significant risk factor. This breed is a crossing of the breeds of Texel (non-dairy sheep) and Flemish sheep (dairy sheep); therefore, the Blessumer breed might have a lower disease resistance [29, 30]. Differences in infection rates between sheep breeds have not yet been studied to investigate whether Blessumer sheep are more often infected.



Table 3. Univariate multilevel analysis of farm-based factors related to *C. burnetii* seropositivity in non-dairy sheep farm residents ( $P < 0.20$ )

Variable	Category	Frequency ( <i>N</i> ) ( <i>N</i> = 261)*	Sero- prevalence (%)	OR (95% CI)
Urbanization†‡§	Moderate or minor urban area	28	67.9	2.00 (0.80–5.04)
	Rural area	242	49.2	Reference
Goat density (number of goats/km <sup>2</sup> excluding own animals in a 5-km radius)†§	<2.9	135	38.5	Reference
	2.9–11.3	67	68.7	3.59 (1.86–6.91)
	≥ 11.4	68	58.8	2.38 (1.18–4.79)
Sheep density (number of sheep/km <sup>2</sup> excluding own animals in 5-km radius)†§	<33.7	133	41.4	Reference
	33.7–79.0	69	53.6	1.68 (0.87–3.25)
	≥ 79.1	68	67.7	2.98 (1.54–5.78)
Cattle density (number of cattle/km <sup>2</sup> in the municipality)†§	< 200.0	240	47.9	Reference
	≥ 200.0	30	76.7	3.20 (1.37–7.51)
Number of Q fever bulk-milk-positive dairy goat or dairy sheep farms in a 10-km radius†§	0	166	45.8	Reference
	1–4	104	59.6	1.78 (1.02–3.11)
Closest Q fever bulk-milk-positive dairy goat or dairy sheep farms (km)§	<5.0	35	62.9	Reference
	5.0–9.9	69	58.0	0.39 (0.14–1.13)
	10.0–14.9	53	41.5	0.87 (0.30–2.54)
	15.0–19.9	41	61.0	0.82 (0.32–2.14)
Year farm started†	≥ 20.0	72	40.3	0.42 (0.16–1.10)
	Before 1990	165	44.2	Reference
Distance between house and pastures	1990 or later	75	61.3	1.97 (1.12–3.48)
	<30 m	127	40.2	Reference
Number of male sheep 2010†	≥ 30 m	103	61.1	2.20 (1.23–3.94)
	<6	130	46.9	Reference
	6–20	56	60.7	1.78 (0.85–3.75)
	>20	41	51.2	1.20 (0.53–2.70)
Zwartbles breed present on farm†	No	16	56.3	1.30 (0.42–4.00)
	Yes	30	63.3	1.75 (0.89–3.42)
Rijnlam breed present on farm	No	228	48.7	Reference
	Yes	7	85.7	5.72 (0.78–42.12)
Blessumer breed present on farm†	No	251	49.4	Reference
	Yes	21	76.2	3.51 (1.25–9.81)
Animals at same pasture simultaneously with sheep	No	237	48.1	Reference
	Cattle	66	59.1	1.30 (0.73–2.33)
	Other	27	18.5	0.21 (0.07–0.66)
Cattle at same pasture but not simultaneously with sheep†	Yes	62	74.2	3.90 (1.74–8.72)
	No	188	42.0	Reference
Straw bedding in the stables	Yes	243	50.2	0.69 (0.40–1.21)
	No	5	60.0	Reference
	No stable	10	50.0	0.31 (0.24–1.68)
How often bedding in stable is refreshed†	Every other day or more	200	53.0	1.77 (0.83–3.76)
	Once or twice a week	47	38.3	Reference
	No stable	10	50.0	1.46 (0.49–4.35)
Air enters stable through door†	Yes	163	46.6	0.64 (0.35–1.18)
	No	79	58.2	Reference
	No stable	10	50.0	0.67 (0.25–1.80)
No farm animals present on farm other than sheep	Yes	73	42.5	0.63 (0.34–1.14)
	No	183	53.6	Reference
Other farm animals present in sheep stables	Yes	164	54.9	1.71 (0.98–3.00)
	No	92	42.4	Reference
Laying hen in stable†	Yes	35	65.7	2.11 (0.88–5.04)
	No	215	47.9	Reference

Table 3 (cont.)

Variable	Category	Frequency (N) (N=261)*	Sero- prevalence (%)	OR (95% CI)
Dairy cattle in stable†	Yes	66	71.2	3.37 (1.76–6.45)
	No	184	42.9	Reference
Type of feed method	By hand/ wheelbarrow	208	48.1	Reference
	Mixer	14	71.4	2.91 (0.92–9.23)
	Shovel	33	48.5	1.02 (0.53–1.97)
Lambing outside†	Yes	27	37.0	0.55 (0.26–1.20)
	No	234	51.3	Reference
Number of yearlings which lambed in 2009†	<40	208	46.6	Reference
	≥40	50	62.0	1.79 (0.89–3.63)
Number dead-born lambs in 2009	<6	49	40.8	Reference
	6–14	93	57.0	1.88 (0.85–4.15)
	15–24	53	41.5	1.09 (0.47–2.50)
	>25	48	54.2	1.69 (0.71–4.05)
Abortion rate 2007, 2008, 2009(%)†	<4 in all three years	195	46.2	Reference
	≥4 in at least one year	51	66.7	2.35 (1.12–4.92)
Afterbirth of normally lambed animal†	Leave in stable or pasture	50	58.0	Reference
		84	47.6	0.64 (0.30–1.36)
	Direct or once a day render bucket	100	51.0	0.72 (0.34–1.53)
	Direct or once a day manure yard	20	30.0	0.31 (0.10–0.97)
	Other			
Farm tenure †	Closed for ewes and rams or only closed for ewes	185	43.2	Reference
	Not closed for ewes and rams	72	65.3	2.37 (1.24–4.54)
Sheep supplied from Groningen†	Yes	26	69.2	2.50 (0.82–7.57)
	No	226	48.2	Reference
Sheep supplied from Noord- Brabant†	Yes	27	63.0	1.93 (0.81–4.58)
	No	225	48.9	Reference
Sheep supplied from Noord- Holland†	Yes	76	59.2	1.67 (0.89–3.15)
	No	176	46.6	Reference
Sheep supplied from Utrecht	Yes	15	73.3	2.69 (0.73–9.86)
	No	237	49.0	Reference
Presence of hygienic locker room	Yes	19	68.4	2.32 (0.81–6.62)
	No	231	48.5	Reference
Presence of disinfection bucket†	Yes	36	61.1	1.80 (0.89–3.65)
	No	214	48.1	Reference

N, Total number of individuals; OR, odds ratio; CI, confidence interval.

\* Not all numbers add up to the total due to missing values.

† Variable included in later multivariate farm-based analyses before manual backward elimination.

‡ Urbanization degree: moderate urban area = 1000–1500 addresses/km<sup>2</sup>; minor urban area = 500–1000 addresses/km<sup>2</sup>; rural area <500 addresses/km<sup>2</sup>.

§ For the geographical data, information was available for all 270 individuals, including the nine people without a farm-based questionnaire.

In the environment of dairy goat farms with a history of abortion waves and of farms having PCR-positive bulk milk, relatively high levels of *C. burnetii* DNA were found [34]. A high goat density in the surrounding area of a participating farm is therefore considered a plausible risk factor for people

living in the vicinity at the time of data collection. This was also demonstrated in several local outbreak investigations in The Netherlands in 2008–2009 [7, 8].

Moreover, several risk factors for *C. burnetii* seropositivity in non-dairy sheep farm residents point to cattle exposure at present or in the past. This might

Table 4. Results of the multivariate logistic regression analysis for the individual characteristics ( $P < 0.10$ ,  $-2LL$ ) in relation to non-dairy sheep farm residents *C. burnetii* seropositivity

Variable	Category	OR (95% CI)
Age (years)	12–19	2.81 (0.85–9.35)
	20–39	1.42 (0.57–3.54)
	40–49	2.29 (1.00–5.24)
	50–59	1.12 (0.50–2.48)
	>60	Reference
Amount of work at farm	Full working week	2.42 (1.13–5.15)
	Up to half a working week	1.23 (0.65–2.33)
	Never/occasionally	Reference
Contact with cattle at own or other farm*	Yes	3.87 (2.13–7.04)
	No	Reference
Worked in cattle sector in the past	Yes	1.79 (1.01–3.18)
	No	Reference

OR, Odds ratio; CI, confidence interval;  $-2LL$ , likelihood ratio test; AIC, Akaike's Information Criterion.

Number of observations used: 266 (AIC = 340.38).

\* See animals at <5 m or touch animals.

Table 5. Results of the multilevel analysis with farm-based characteristics ( $P < 0.10$ ) as independent factors in relation to non-dairy sheep farm residents *C. burnetii* seroprevalence

Variable	Category	OR (95% CI)
Goat density (number of goats/km <sup>2</sup> excluding own animals in a 5-km radius)	<2.9	Reference
	2.9–11.3	1.60 (0.75–3.43)
	≥11.4	3.80 (1.67–8.65)
Year farm started	Before 1990	Reference
	1990 or later	3.97 (1.79–8.82)
Blessumer breed present on farm	Yes	5.19 (2.36–11.41)
	No	Reference
Cattle at same pasture but not simultaneously with sheep	Yes	5.14 (2.17–12.19)
	No	Reference
How often bedding in stable is refreshed	Every other day or more	3.24 (1.49–7.07)
	Once or twice a week	Reference
	No stable	8.91 (2.17–36.68)
Air enters stable through door	Yes	0.46 (0.23–0.92)
	No	Reference
	No stable	8.91 (2.17–36.68)
Dairy cattle present during stabling period of sheep	Yes	3.33 (1.17–9.46)
	No	Reference
Lambing outside	Yes	0.34 (0.14–0.86)
	No	Reference
Sheep supplied from Groningen	Yes	4.17 (1.59–10.97)
	No	Reference
Sheep supplied from Noord-Holland	Yes	3.93 (1.74–8.90)
	No	Reference

OR, Odds ratio; CI, confidence interval; QIC, quasi-likelihood under the independence model criterion.

Number of observations used: 212. Number of levels used: 107 (QIC = 232.9560).

suggest that cattle were partially responsible for the infections observed in the sheep farm residents. In a previous study in farmers (all farm types) contact with cattle was also described as a risk [27]. A recent

published review including worldwide studies, suggested a higher seroprevalence of *C. burnetii* in cattle compared to goat and sheep [35]. In The Netherlands, a prevalence of 78.6% for antibodies

Table 6. Results of the multilevel analysis with individual and farm-based characteristics ( $P < 0.10$ ) as independent factors in relation to non-dairy sheep farm residents *C. burnetii* seroprevalence

Variable	Category	OR (95% CI)
Age (years)	12–19	0.96 (0.29–3.21)
	20–39	1.96 (0.56–6.90)
	40–49	2.43 (0.98–6.04)
	50–59	1.54 (0.63–3.78)
	>60	Reference
Contact with cattle at own or other farm*	Yes	2.32 (1.02–5.29)
	No	Reference
Worked in cattle sector in the past	Yes	3.98 (1.71–9.25)
	No	Reference
Goat density (number of goats/km <sup>2</sup> excluding own animals in a 5-km radius)	<2.9	Reference
	2.9–11.3	1.11 (0.46–2.68)
	≥11.4	5.86 (1.81–18.95)
Year farm started	Before 1990	Reference
	1990 or Later	3.67 (1.45–9.31)
Blessumer breed present on farm	Yes	4.49 (1.59–12.65)
	No	Reference
Cattle at same pasture but not simultaneously with sheep	Yes	5.77 (2.29–14.56)
	No	Reference
How often bedding in stable is refreshed	Every other day or more	4.58 (1.69–12.37)
	Once or twice a week	Reference
	No stable	8.34 (1.71–40.60)
Air enters stable through door	Yes	0.47 (0.21–1.01)
	No	Reference
	No stable	8.34 (1.71–40.60)
Dairy cattle present during stabling period of sheep	Yes	2.69 (0.81–8.95)
	No	Reference
Lambing outside	Yes	0.33 (0.12–0.92)
	No	Reference
Sheep supplied from Groningen	Yes	5.05 (1.73–14.69)
	No	Reference
Sheep supplied from Noord-Holland	Yes	3.63 (1.27–10.33)
	No	Reference

OR, Odds ratio; CI, confidence interval; QIC, quasi-likelihood under the independence model criterion.

Number of observations used: 208. Number of levels used: 105 (QIC = 219.1157).

\* See animals at <5 m or touch animals.

in cattle bulk tank milk was found, confirming widespread circulation of the bacterium in cattle [36]. To further assess the risk for human infection from cattle, a similar study addressing the seroprevalence and risk factors in dairy cattle farm residents is being finalized in The Netherlands. A role for cattle in the human infections observed in the current sheep farm study, is also supported by the fact that the high seroprevalence in sheep farm residents does not seem to correspond with the low sheep seroprevalence at the participating farms (<2%). The role of specific activities with sheep for the infection risk was presumably relatively small, although not absent taking into account the significant association between human and sheep seroprevalence at the participating

non-dairy farms. Whether sheep themselves are at increased risk for infection because of contact with cattle or nearby goat populations is currently under investigation. In The Netherlands, a dominant *C. burnetii* genotype was identified in humans, goats, and sheep throughout the entire affected area; the genotype found in cattle appeared to be different [37, 38].

Based on the results of the present study, some recommendations can be made. First, we want to elucidate the transmission cycle between different species of ruminants and farm residents; strains from goat, sheep, cattle, and sheep farm residents could be subtyped and compared. Second, more research is needed to investigate whether the Blessumer breed is more often infected compared to other breeds. Third,

in this study a high seroprevalence in spouses was found (36·3% non-dairy farm spouses, 50·0% dairy farm spouses). Therefore, we emphasize the importance of the advice that pregnant women should avoid contact with sheep during the lambing season, and that they should avoid contact with birth products of sheep. Currently, the Dutch Health Council is preparing an advice about vaccination of high-risk professionals, including several farm populations. For this advice, they also will take into account the results of this study.

### Limitations

The study of non-dairy sheep farms had a low response rate of 9·1%. As reported by several farmers not willing to participate, sheep were outside when the request to participate was made, and it would be too labour-intensive to collect about 60 sheep for blood sampling. In addition, this part of the sheep industry was not affected by the implemented control measures, mainly targeted at farms with dairy sheep and dairy goats. Therefore, non-dairy sheep farmers might be less motivated to participate compared to the small dairy sheep sector, which had a response rate of 38%.

Except for differences in sheep density in the surroundings and the number of sheep on their farms, participating and non-participating non-dairy sheep farms appeared to be comparable. As both factors were not related to seropositivity, this selective response is not thought to be of influence on the study results, which are therefore considered representative for the Dutch professional non-dairy sheep sector.

At 79% of the 119 participating non-dairy farms both the farmer and partner participated in the study. Therefore, results for the farmers and partners are considered representative of the group of farmers/partners at the participating farms. It was not registered how many children aged  $\geq 12$  years lived at the participating non-dairy farms, and we cannot be absolutely sure that the participating children were representative of all children in this age category.

### CONCLUSION

This study demonstrates that *C. burnetii* infection is common in individuals living and/or working at a sheep farm in The Netherlands. Except for their sheep, the risk also seems dictated by contact with

cattle at present or in the past and by nearby goat populations.

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### DECLARATION OF INTEREST

None.

### REFERENCES

1. **Maurin M, Raoult D.** Q fever. *Clinical Microbiology Reviews* 1999; **12**: 518–553.
2. **Marrie TJ, et al.** Truckin' pneumonia—an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. *Epidemiology and Infection* 1989; **102**: 119–127.
3. **Gonder JC, et al.** Cynomolgus monkey model for experimental Q fever infection. *Journal of Infectious Diseases* 1979; **139**: 191–196.
4. **Raoult D, et al.** Q fever 1985–1998. Clinical and epidemiologic features of 1,383 infections. *Medicine (Baltimore)* 2000; **79**: 109–123.
5. **van der Hoek W, et al.** Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in The Netherlands: from acute to chronic infection. *Euro-surveillance* 2012; **17**: pii=20059.

6. **van der Hoek W, et al.** Q fever in the Netherlands: an update on the epidemiology and control measures. *Eurosurveillance* 2010; **15**: pii=19520.
7. **Karagiannis I, et al.** Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiology and Infection* 2009; **137**: 1283–1294.
8. **Schimmer B, et al.** The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases* 2010; **10**: 69.
9. **Schimmer B, et al.** Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. *Eurosurveillance* 2009; **14**: pii=19210.
10. **van der Hoek W, et al.** Human Q fever and non-dairy farm [in Dutch]. National Institute of Public Health and Environment, 2010. Report No.: 2010/02.
11. **Koene RP, et al.** A Q fever outbreak in a psychiatric care institution in The Netherlands. *Epidemiology and Infection* 2011; **139**: 13–18.
12. **Whelan J, et al.** Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009. *Epidemiology and Infection* 2012; **140**: 858–864.
13. **Gilsdorf A, et al.** Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. *Epidemiology and Infection* 2008; **136**: 1084–1087.
14. **Hellenbrand W, Breuer T, Petersen L.** Changing epidemiology of Q fever in Germany, 1947–1999. *Emerging Infectious Diseases* 2001; **7**: 789–796.
15. **Porten K, et al.** A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infectious Diseases* 2006; **6**: 147.
16. **Medić A, et al.** Q fever epidemic among employees in a factory in the suburb of Zadar, Croatia. *Croatian Medical Journal* 2005; **46**: 315–319.
17. **Dupuis G, et al.** An important outbreak of human Q fever in a Swiss Alpine valley. *International Journal of Epidemiology* 1987; **16**: 282–287.
18. **Starnini G, et al.** An outbreak of Q fever in a prison in Italy. *Epidemiology and Infection* 2005; **133**: 377–380.
19. **Manfredi Selvaggi T, et al.** Investigation of a Q-fever outbreak in northern Italy. *European Journal of Epidemiology* 1996; **12**: 403–408.
20. **Wegdam-Blans MCA, et al.** Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. *Clinical and Vaccine Immunology* 2012; **19**: 1110–1115.
21. **Hussain-Yusuf H, et al.** An analysis of Q fever patients 6 years after an outbreak in Newport, Wales, UK. *QJM: an International Journal of Medicine* 2012; **105**: 1067–1073.
22. **Wegdam-Blans MCA, et al.** Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. *Journal of Infection* 2012; **64**: 247–259.
23. **Schimmer B, et al.** Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. *Epidemiology and Infection* 2012; **140**: 27–35.
24. **Hogema BM, et al.** *Coxiella burnetii* infection among blood donors during the 2009 Q-fever outbreak in The Netherlands. *Transfusion* 2012; **52**: 144–150.
25. **Macellaro A, Akesson A, Norlander L.** A survey of Q-fever in Sweden. *European Journal of Epidemiology* 1993; **9**: 213–216.
26. **Cisak E, et al.** Prevalence of antibodies to *Coxiella burnetii* among farming population in eastern Poland. *Annals of Agricultural and Environmental Medicine* 2003; **10**: 265–267.
27. **Thomas DR, et al.** The risk of acquiring Q fever on farms: a seroepidemiological study. *Occupational and Environmental Medicine* 1995; **52**: 644–647.
28. **Schimmer B, et al.** Seroprevalence and Risk Factors for *Coxiella burnetii* (Q Fever) Seropositivity in Dairy Goat Farmers' Households in The Netherlands, 2009–2010. *PLoS One* 2012; **7**: e42364.
29. **Rauw WM, et al.** Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* 1998; **56**: 15–33.
30. **van den Brom R, et al.** Demography of Q fever seroprevalence in sheep and goats in The Netherlands in 2008. *Preventive Veterinary Medicine* 2012.
31. **McCaughey C, et al.** Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. *Zoonoses Public Health* 2008; **55**: 189–194.
32. **Bölske G, et al.** Bovine tuberculosis in Swedish deer farms: epidemiological investigations and tracing using restriction fragment analysis. *Veterinary Record* 1995; **136**: 414–417.
33. **Mansley LM, et al.** Early dissemination of foot-and-mouth disease virus through sheep marketing in February 2001. *Veterinary Record* 2003; **153**: 43–50.
34. **de Bruin A, et al.** Detection of *Coxiella burnetii* DNA on small-ruminant farms during a Q fever outbreak in the Netherlands. *Applied and Environmental Microbiology* 2012; **78**: 1652–1657.
35. **Guatteo R, et al.** Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. *Veterinary Microbiology* 2011; **149**: 1–16.
36. **Muskens J, et al.** Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. *Veterinary Record* 2011; **168**: 79.
37. **Roest HIJ, et al.** Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. *Emerging Infectious Diseases* 2011; **17**: 668–675.
38. **Tilburg JJHC, et al.** Epidemic genotype of *Coxiella burnetii* among goats, sheep, and humans in the Netherlands. *Emerging Infectious Diseases* 2012; **18**: 887–889.