High *Coxiella burnetii* Seroconversion Rate in Veterinary Students, the Netherlands, 2006–2010

Marit M.A. de Lange, Wim van der Hoek, Peter M. Schneeberger, Arno Swart, Dick J.J. Heederik, Barbara Schimmer, Inge M. Wouters

Author affiliations: National Institute for Public Health and the Environment, Bilthoven, the Netherlands (M.M.A. de Lange, W. van der Hoek, A. Swart, B. Schimmer); Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands (P.M. Schneeberger); Utrecht University, Utrecht, the Netherlands (D.J.J. Heederik, I.M. Wouters)

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We examined *Coxiella burnetii* seroconversion rates by measuring *C. burnetii* IgG among 2 cohorts of veterinary students. During follow-up of 118 seronegative veterinary students, 23 students seroconverted. Although the clinical importance of the presence of antibodies is unknown, veterinary students should be informed about the potential risks for Q fever.

Q fever is caused by the bacteria *Coxiella burnetii* and can manifest as acute or chronic illness. Veterinarians who care for livestock are prone to *C. burnetii* infection (*1*,*2*). A high seroprevalence among veterinary students has been reported (*3*–*5*). However, the incidence of Q fever and associated risk factors during veterinary training are still unknown. We conducted a longitudinal study at the Faculty of Veterinary Medicine of Utrecht University (FVMUU), Utrecht, the Netherlands, in which we followed incoming, seronegative veterinary students and investigated potential associated factors for seroconversion.

Veterinary students who started in 2006 or 2008 at FVMUU were invited to participate. After obtaining written informed consent, we collected blood samples, and participants completed a baseline questionnaire. From participants who began at FVMUU in 2006 (cohort 2006), ≤2 additional blood samples and follow-up questionnaires were obtained in 2008 and 2010. Students who started in 2008 (cohort 2008) provided 1 follow-up blood sample and 1 follow-up questionnaire in 2010.

Serum samples were tested for IgG against phase I and II of *C. burnetii*, using an indirect immunofluorescence assay as previously described (*3*). Those samples with IgG phase I or II IgG ≥1:32 were classified as *C. burnetii* seropositive. Seroconversion was defined as the change observed in a participant who was IgG seronegative at baseline and seropositive in a follow-up sample.

We determined differences in demographics and past animal exposure between seropositive and seronegative participants at baseline. Risk factors for seroconversion were estimated by using univariable logistic regression analyses through generalized estimating equations models (Appendix, https://wwwnc.cdc.gov/EID/article/26/12/20-0063-App1.pdf).

At the beginning of their veterinary training, 447 students were invited to participate in the study. Of those, 131 participated, of whom 13 (10%) were *C. burnetii* IgG seropositive at baseline. Students who were seropositive at baseline were more likely to have lived on a farm and to have had contact with cattle and poultry (Appendix Table 1).

Of the 118 participants seronegative at baseline, 78 started their training in 2006 and 40 in 2008 (Figure). Of those students, 23 seroconverted during the follow-up period of 362 person-years, translating to an incidence of 0.06/person-year. Of the 17 seroconversions in cohort 2006, 11 occurred between baseline and the

	2006	2008	2010
No. in 2006 cohort	78	77	73
Questionnaire no., %	77/78 (99)	77/77 (100)	71/73 (97)
Blood no., %	78/78 (100)	72/77 (94)	63/73 (86)
Seropositive no., %	0/78 (0)	11/77 (15)	17/63 (27)
No. in 2008 cohort		40	40
Questionnaire no., %		39/40 (98)	38/40 (95)
Blood no., %		40/40 (100)	40/40 (100)
Seropositive no., %		0/40/(0)	6/40 (15)

Figure. Follow-up timeline illustrating number and percentages of seronegative participants at baseline, per follow-up moment, in study of *Coxiella burnetii* seroconversion rate in veterinary students, the Netherlands, 2006–2010. The 17 seropositive students in 2010 include the 11 students who already seroconverted during 2006–2008 and were censored from risk factor analysis in 2010.

Table. Characteristics from follow-up questionnaire in association with *Coxiella burnetii* seroconversion among 118 veterinary students seronegative at baseline, the Netherlands*

Characteristic	Odds ratio (95% CI)	p value
Age group, y		
≤20	Referent	
21	0.9 (0.2–3.5)	0.85
≥22	1.3 (0.4–4.2)	0.69
Sex		
M	Referent	
F	0.7 (0.2–2.3)	0.53
Regular exposure to cigarette smoke		
Yes	1.1 (0.4–2.8)	0.81
No	Referent	
Living on a farm with cattle		
Yes	ND	
No	ND	
Living on a farm with sheep or goats		
Yes	6.2 (1.4–28.1)	0.02
No	Referent	
Living on a farm with pigs		
Yes	ND	
No	ND	
Living on a farm with chickens		
Yes	3.0 (0.3–35.0)	0.39
No	Referent	
Regular contact with cattle outside veterinary training		
Yes	0.3 (0.1–2.7)	0.31
No	Referent	
Regular contact with goats outside veterinary training		
Yes	0.6 (0.1–3.8)	0.56
No	Referent	
Regular contact with horses outside veterinary training		
Yes	0.7 (0.3–1.7)	0.40
No	Referent	
Regular contact with pigs outside veterinary training		
Yes	ND	
No	ND	
Regular contact with chickens outside veterinary training		
Yes	0.5 (0.1–3.8)	0.50
No	Referent	
Regular contact with sheep outside veterinary training		
Yes	4.4 (1.2–16.7)	0.03
No	Referent	
History of performing animal nursing on farm where they lived		
Yes	3.6 (0.9–14.3)	0.07
No	Referent	
History of working with straw or hay on farm where they lived		
Yes	6.4 (1.6–26.1)	<0.01
No	Referent	
History of working with fertilizers on farm where they lived		
Yes	3.2 (0.5–19.6)	0.21
No	Referent	
History of performing plant nursing on farm where they lived		
Yes	3.1 (0.3–33.5)	0.35
No	Referent	
No. years after study start†		
2	Referent	
4	1.0 (0.3–2.9)	0.96
Cohort‡		
2006	Referent	
2008	0.7 (0.3–2.0)	0.56
Chosen specialization during veterinary training		
Individually kept animals	Referent	
Veterinary public health or farm animals	1.6 (0.5–5.0)	0.38

*ND, not determined because of low numbers.
†Only adjusted for cohort.
‡Only adjusted for number of years after the study.

first follow-up, and 4 occurred between the first and second follow-up (Appendix Table 2). None of the seroconverted participants reported a diagnosis of acute Q fever from a general practitioner or medical specialist, suggesting all cases were mild or asymptomatic. In addition, no participants had serologic indication of a chronic infection. Of the 20 investigated characteristics, "living on a sheep or goat farm," "having contact with sheep outside [veterinary] training," and "working with hay, straw, silage grass, or animal feed" outside FVMUU increased the odds of seroconversion (Table).

We were not able to identify education-related potential risk factors, such as courses taken, for 2 reasons. First, the curriculum changed during our study, so participants from the 2006 and 2008 cohorts took different courses, causing low power in the analysis. Second, within each cohort, little variation occurred in courses taken. Another limitation of this study is our assumption of a constant risk for *C. burnetii* exposure during the study period. Students seem to have been at higher risk for infection in the first 2 study years, although we cannot draw definite conclusions from this small group of students.

Identified risk factors for seroconversion were not education-related. Proximity to (aborting) small ruminants, such as goats and sheep, was a risk factor in an outbreak in the Netherlands (*6*). Veterinary students have a high prevalence of animal contacts outside their education (*7*). In addition, contact with hay, straw, silage grass, or animal feed, is a known risk factor for human Q fever (*8*). A major outbreak of acute Q fever occurred in the Netherlands during 2007–2010 (*9*), and some students might have contracted the infection then, although increased seroprevalence of Q fever in veterinary students before that outbreak has been reported (*3*).

In conclusion, we found a considerable *C. burnetii* seroconversion rate among veterinary students. Although the clinical importance of the presence of antibodies is unknown, students should be advised at the beginning of their education about potential risks and instructed to seek care if they experience symptoms of acute or chronic Q fever infection.

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About the Author

Dr. de Lange is an epidemiologist at the National Institute for Public Health and the Environment. She conducted her PhD research on Q fever. Her other research interests include respiratory infections, such as influenza and respiratory syncytial virus.

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Address for Correspondence: Marit M.A. de Lange, National Institute for Public Health and the Environment, Centre for Infectious Disease Control Netherlands, Antonie van Leeuwenhoeklaan 9, 3721 MA, Bilthoven, the Netherlands; email: marit.de.lange@rivm.nl

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Appendix

Methods

The Study

The Medical Ethical Commission of the University Medical Centre Utrecht approved the study protocol (no. 06/169). Veterinary students who started in 2006 or 2008 (around 225 each year) at the Faculty of Veterinary Medicine of Utrecht University (FVMUU), which is the only Dutch veterinary institution, were invited to participate. After obtaining written informed consent, we collected a blood sample at study start and participants completed a baseline questionnaire. From participants who started at the FVMUU in 2006 (cohort 2006), up to 2 additional blood samples and follow-up questionnaires were obtained in 2008 and 2010. Students who started in 2008 (cohort 2008) provided 1 follow-up blood sample and follow-up questionnaire in 2010.

Questionnaires

Both the baseline and follow-up questionnaires included questions about animal contact, living situation, personal health situation, and smoking habits before and during the study period. The follow-up questionnaires also included questions about focus of study.

Serologic Analysis

Serum samples were tested for IgG antibodies against phase I and II of *C. burnetii*, using an indirect immunofluorescence assay as previously described (*1*). To avoid batch differences, all samples were tested at the end of the study in 1 batch. Those with IgG phase I or II antibodies $\geq$1:32 were classified as *C. burnetii* seropositive. Seroconversion was defined as a participant who was IgG seronegative at baseline and seropositive in 1 of the follow-up samples.

Participants with an IgG phase I titer of ≥1:1024 had a serologic indication for chronic Q fever infection (*2*).

Statistical Analysis

All data were analyzed with SAS, version 9.4 (SAS Institute Inc., https://www.sas.com/en_us/home.html). First, differences in demographics and past animal exposure characteristics between seropositive and seronegative participants at baseline were determined (Appendix Table 1). To estimate seroconversion rate and possible associated factors for seroconversion during follow-up, data from seronegative participants with at least one follow-up sample were used. The univariable logistic regression analyses were performed with generalized estimating equations models with an exchangeable correlation matrix. These models were used to take into account correlations between the repeated measurements of serostatus within the same subject (*3*). Participants' data were censured for the times after they were tested *C. burnetii* seropositive. The data from the two cohorts were analyzed together because the datasets were too small to analyze them separately. The FVMUU starting year (cohort) and the number of years after the study's beginning were always included as covariates in the model. Investigated characteristics were animal-related exposure outside the study, living situation, smoking habits, study duration, cohort, and chosen area of study; in total, we investigated 20 characteristics. Associations were considered significant at confidence level of $\alpha < 0.05$. All univariable associated characteristics were highly interrelated ($p < 0.05$ in Fisher exact test). Therefore, multivariable logistic analysis with generalized estimating equations was not possible.

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Appendix Table 1. Baseline questionnaire characteristics of 2 cohorts of veterinary students, the Netherlands*

Characteristic	Median or no./N (%) Seronegative participants at baseline, 2006 or 2008, n = 118†	Seropositive participants at baseline, 2006 or 2008, n = 13	p value
Age, y	19	18	0.24‡
BMI	21	21	0.87‡
Gender			
M	18/116 (16)	3/13 (23)	0.44
F	98/116 (84)	10/13 (77)	
Smoking status			
Never smoked	109/115 (94)	13/13 (100)	1.00
Past smoker	3/115 (3)	0/13 (0)	
Current smoker	3/115 (3)	0/13 (0)	
Size of place of origin			
Village (<15,000 inhabitants)	44/116 (38)	7/13 (54)	0.45
Town (15,000–80,000 inhabitants)	47/116 (40)	5/13 (38)	
City (>80,000 inhabitants)	25/116 (22)	1/13 (8)	
Ever lived on a farm			
Yes	11/116 (9)	7/13 (54)	<0.01
No	105/116 (91)	6/13 (46)	
Regular contact with cattle before start of FVMUU			
Yes	19/116 (16)	9/13 (69)	<0.01
No	97/116 (84)	4/13 (31)	
Regular contact with goats before start of FVMUU			
Yes	18/116 (16)	5/13 (38)	0.06
No	98/116 (84)	8/13 (62)	
Regular contact with sheep before start of FVMUU			
Yes	20/116 (17)	5/13 (38)	0.13
No	96/116 (83)	8/13 (62)	
Regular contact with poultry before start of FVMUU			
Yes	31/116 (27)	8/13 (62)	0.02
No	85/116 (73)	5/13 (38)	
Regular contact with horses before start of FVMUU			
Yes	65/116 (56)	7/13 (54)	1.00
No	51/116 (44)	6/13 (46)	
Regular contact with pigs before start of FVMUU			
Yes	13/116 (11)	4/13 (31)	0.07
No	103/116 (89)	9/13 (69)	

* BMI, Body Mass Index; FVMUU, Faculty of Veterinary Medicine of Utrecht University; n, Number; N, Total number.
†Two seronegative participants at baseline did not fill out a questionnaire.
‡P value of age and BMI were determined by Fisher Exact test. All other p values shown were determined by Kruskal Wallis test.

Appendix Table 2. Distribution of 2006 cohort, 78 seronegative students at baseline with at least one follow-up sample

Result at 2008 follow-up	Result at 2010 follow-up	No. of students
Positive	Positive	7
Negative	Negative	44
Positive	Negative	3
Negative	Positive	3
Positive	Not tested	1
Negative	Not tested	14
Not tested	Positive	2
Not tested	Negative	4