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Risk Factors For Q Fever Seroconversion In A Cohort Of Veterinary Students: A Comparative Analysis

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Risk factors for Q-fever seroconversion in a cohort of veterinary medical students: a comparative study

INTRODUCTION

Coxiella burnetii (C. burnetii), the causative agent of the zoonosis Q fever, is an extremely infectious and environmentally-persistent intracellular bacterium (Raoult et al., 2005; Kersh et al., 2013). Human infection was first described in 1937 among abattoir workers in Australia who experienced fever, severe headache, rigors, myalgia, arthralgia, and rash (Parker et al., 2006). Today, *C. burnetii* has been found in nearly every country worldwide and is classified by CDC as a potential bioterrorism agent due to its extreme infectivity (CDC, 2014; Parker et al., 2006).

Ruminants are considered the primary reservoirs associated with human infection, though the bacterium is found in a variety of domestic and wild animal species (van der Hoek, 2012). Infected animals shed bacteria into the environment through biological materials such as birthing products, milk, and urine (Parker et al., 2006). Small ruminants, goats and sheep in particular, seem to play an important role in the transmission of Q fever due to their synchronized parturitions which may release massive amounts (billions) of bacteria within a relatively short time (van der Hoek, 2012; Maurin et al., 1999). The risk of infection from animals that intermittently shed bacteria is substantially lower (van der Hoek, 2012). Human exposure is primarily via inhalation of aerosolized bacteria (Jones et al., 2006). Dust contaminated with *C. burnetii* may accumulate and re-aerosolize days, months, or years after its initial release (van der Hoek, 2012). The importance of environmental transmission was seen during an unprecedented Q fever epidemic in the Netherlands between 2007-2010, after spatial models linked human cases to abortion storms on nearby dairy goat and dairy sheep farms (van der Hoek, 2010).

It is estimated, but debated, that up to 60% of acute infections are asymptomatic (de Rooij et al., 2012). If symptomatic, human cases generally present as a non-specific febrile illness and are often misdiagnosed (Parker et al., 2006). Infection may resolve spontaneously or become chronic with no prior indication of acute illness. Severe cases of acute Q fever may result in pneumonia, hepatitis, or myocarditis (van der Hoek, 2012; Parker et al., 2006). A percentage of acute infections may progress into chronic Q fever, a potentially fatal disease, or a debilitating post-Q fever fatigue syndrome (van der Hoek, 2012). In general, laboratory analysis of antibodies against phase I and phase II antigens of *C. burnetii* is required to confirm a suspected infection (Teunis et al., 2013)

Although the Dutch epidemic highlighted the public health importance of Q fever, risk of infection is largely considered an occupational hazard. Following the epidemic's peak, studies in the Netherlands found 87.2% of dairy cattle farmers and 73.9% of dairy goat farmers to be seropositive (Schimmer et al., 2012, 2014). Epidemiological data from the Netherlands and elsewhere have consistently identified increasing age, male gender, and active smoking to be associated with seropositivity (van der Hoek, 2012). Reported seroprevalence figures in the general population are substantially lower. A 2006 study in the Netherlands found 2.4% of the general population to be seropositive, a figure comparable to the 3.1% seropositivity rate reported by a U.S. study of NHANES data around the same time (de Rooij et al., 2012; Vest et al., 2013).

The interpretation of Q fever seroprevalence data is challenging for two reasons. First, resolved cases are assumed to be susceptible to re-infection, though a degree of immunity has been suggested (van der Hoek, 2012). Second, antibody clearance rates are subject to inter-individual variability with evidence that titers may be maintained for extended periods of time (Teunis et al, 2013). For instance, analysis of sera from US Army Veterinarians between 1989-2008 found 17% of late career professionals to be seropositive, yet only 3.6% of veterinarians seroconverted during the study period (Vest et al., 2013). For these reasons, seroconversion endpoints are preferred over seropositivity in investigations of risk factors for Q fever infection though seroconversion information is often not available.

The risk to veterinary students, who arguably have similar exposure profiles as veterinarians, is not well characterized. Intuitively, the populations for which age, male gender, and smoking are significant risk factors differ fundamentally from the typical veterinary student population. In 2000, Valencia et al. found a significantly higher seroprevalence in fifth year students (16.8%) in comparison to first year students (4.0%), and inferred a gradual increase in seropositivity during the course of study (Valencia et al., 2000). In a similar seroprevalence study, Rooij et al. found study direction (livestock versus companion animals), advanced year of study, experiencing a zoonotic disease since starting school, and ever living on a ruminant farm as significant risk factors (Rooij et al., 2012).

To our knowledge, this is the first study of Q fever *seroconversion* in a cohort of veterinary students. In contrast to recent epidemiological studies in the Netherlands that focused on highly exposed populations such as livestock workers on known infected farms, this study aims to characterize the risk of direct-animal contact in a comparatively less-exposed group. By following incoming, seronegative students through their veterinary program, we aim to understand the academic and extracurricular risk factors specific to their experience. We anticipate that results could be important for creating awareness among students and faculty on the risk for Q fever.

METHODS

Study design and population

All students beginning veterinary school at Utrecht University in 2006 (N=225) were invited to participate in the Veterinarians' Health Study with the purpose of assessing the occupational health risks of increased exposure to allergens and infectious and non-infectious agents associated with veterinary work and education. Utrecht University's Veterinary Medical School (VMS) is the only veterinary program in the Netherlands and attracts students throughout the country. Recruitment methods included informational class presentations and mailed brochures. Students were enrolled in the study following receipt of informed consent, completion of a baseline blood sample collected at a central location, and submission of an online questionnaire within the first two months of veterinary study. Non-responders were reminded a maximum of two times. Follow-up periods occurred at two-year intervals (2008 and 2010) at which points students were asked to re-submit blood samples and questionnaires (Figure 1).

Questionnaire

Baseline and follow-up questionnaires were used to collect information on past and recent putative risk factor exposures, occurring both inside and outside of the veterinary study curriculum. Questions at baseline focused on demographic and behavioral characteristics (i.e. age, gender, smoking status), health status and relevant medical history (i.e. self-rated health, prior zoonotic diseases) and assessed historical exposures to livestock, domestic animals, farms, and any prior veterinary schooling.

At follow-up periods, changes in smoking, housing, and health status were addressed. Information on all completed coursework was obtained, used as a proxy for animal contact during the course of study. Questions pertaining to exposures outside VMS included contact with specific animals, zoonotic diagnoses including Q fever, and changes in housing. All animal contact questions were defined as “recent regular” contact outside of VMS (at least one direct-contact interaction per week since the previous follow-up interval). The variable “recently lived on a farm” pertains to the time frame starting two years before baseline assessment through the duration of the study. It differs from “current housing” in that it captures farm exposures that students may have during weekends or extended breaks away from university. Importantly, the animal and farm contact questions capture exposures outside academia while coursework captures exposures associated with veterinary school.

Serology

For the present study, the stored baseline and follow-up serum samples were screened for IgG and IgM antibodies against phase I and phase II of *C. burnetii* using a commercial immunofluorescence assay (IFA), as per the manufacturer’s instructions (Focus Diagnostics). All IFA tests were performed at the Regional Laboratory of Medical Microbiology and Infection Control, of the Jeroen Bosch Hospital in ‘s Hertogenbosch.

Sera were initially analyzed for phase I and phase II IgG antibodies in a two-fold dilution sequence beginning with 1:32 and ending at 1:4096. Samples with phase I or phase II IgG antibody titers ≥ 1:32 were defined as seropositive. IgG levels were determined to be the best indication of an infection during the follow-up interval as they are known to increase a few weeks post-infection and remain detectable for years, or even throughout life (Rooij, Risk Factors). For analysis, seroconversion was defined as the first seropositive result after a prior negative result.

Statistical analysis

At baseline, students’ historical exposures and seropositivity status were compared using Pearson chi-square or Fisher’s exact test. Seropositive students were excluded from further analysis given that seroconversion was the primary outcome of interest. All seronegative students who completed questionnaires at baseline, and completed serology and a questionnaire during at least one follow-up period, were included in seroconversion analyses.

All exposure variables pertained to recent exposures, with the exception of "number of years lived on a farm," which was included to account for a possible degree of immunity from a previous asymptomatic infection. An additional categorical variable representing the year at which follow-up occurred was included due to potential environmental exposure following the onset of the Dutch epidemic.

Exposure characteristics of seroconverted and non-seroconverted students were analyzed by follow-up year. Spearman correlations were calculated for predictors to explore relationships between variables. Bivariate and multivariate generalized linear mixed models were developed, accounting for the repeated measures of some students who attended both follow-ups. Predictors associated with seroconversion ($p<0.3$) in the bivariate analysis were included in the multivariate models. Forward manual selection, followed by backward manual selection, was performed to obtain the final parsimonious model.

A random forest method was selected as a secondary analytical tool. Since random forest does not account for repeated measures, an additional covariate was added. Unlike regression, random forest makes no underlying assumptions about the functional form of the model or distribution of the data. Instead, an ensemble of classification trees is constructed from random subsets of the data, using randomly restricted and selected predictors for each split in the tree (Strobl et al., 2008, 2009). In this aspect, random forests are able to examine the contribution of each predictor even when the effect of one is likely to be overshadowed by more significant competitors in regression models (Strobl et al., 2009). The algorithm predicts the outcome (seroconversion) of an individual based upon the individual's predictors. The relative importance of a predictor is calculated by random permutations of the data for that variable while all others are left unchanged. If the variable has little bearing on prediction it is considered to be of low importance, and vice versa. Model diagnostics include overall prediction accuracy, sensitivity and specificity.

Analyses were performed using SAS 9.3 (SAS Institute, Cary, NC) and R software version 2.9.0 (R-project, Vienna, Austria) *randomForest* package (RF algorithm by L. Breiman and A. Cutler).

RESULTS

Study population

A total of 113 students completed baseline questionnaires and serology in 2006, of which 13 (11.5%) were seropositive. At baseline, seropositivity was found to be significantly associated with a greater number of years lived on a farm and ever having regular contact with goats or bovines (Table 1).

Of the 100 seronegative students at baseline, 67 participated in at least one follow-up and were subsequently included in the seroconversion analyses. The 33 students lost to follow-up included a greater percentage of males, but were otherwise comparable to those who attended follow-up. The cohort was a median of 19 years old, largely female (85.1%), non-smoking (97.0%), had never lived on a farm (88.1%), and never had regular contact with sheep (79.1%), bovines (80.6%), or goats (85.1%) (Table 2). No students reported a previous Q fever infection, including during the study duration.

At the first follow-up in 2008, 13.1% of 61 formerly seronegative students had seroconverted. Those seropositive included 3/9 (33%) of participating males and 4/10 (40%) of students who recently lived on a farm, had recent contact with sheep, or had completed two elective courses. Sixty percent (3/5) of students with more than 10 years living on a farm seroconverted, though nearly all also reported recently living on a farm. At the second follow-up in 2010, 10.2% of formerly negative students seroconverted, which included 1/5 (20%) of students recently living on a farm and 2/7 (29%) who completed both elective courses (Table 3).

Little variation in academic curriculum was found with the exception of five elective courses: “pain management,” “comparative ethology,” “evolution,” “history of veterinary medicine,” and “pre/postnatal care of sheep and lambs.” Upon bivariate analysis only “pain management” and “pre/postnatal care of sheep and lambs” were found independently associated with seroconversion and thus were the only coursework included in analyses. Although initially analyzed individually, the courses were correlated with each other and subsequently combined into the additive variable, “number of completed elective courses.”

Bivariate results

Bivariate analysis of the combined follow-up data showed significant associations ($p<0.05$) between seroconversion and the number of years living on a farm (>10 years OR 6.60, (1.29, 33.81)), recently living on a farm (OR 5.44, (1.49,19.85)), having completed the “pre/post natal sheep and lamb" course (OR 5.22, (1.08, 25.16)), and reporting recent contact with sheep (OR 6.11, (1.65, 22.67)). The additive variable representing the number of elective courses was also significant. Students who took both the "pain" and "pre/postnatal" courses were significantly more likely to seroconvert (OR 8.45, (1.48, 48.25)) than those who did not complete either (Table 4).

Multivariate results

According to the inclusion criteria of $p<0.3$ in bivariate analysis, the variables gender, number of years lived on a farm, recently lived on a farm, recent sheep contact, and number of completed elective courses, were incorporated into backward and forward selection models. Despite strong correlations, the two farm variables were both initially included to investigate the possibility of protective effects from historical farm exposures. The final parsimonious model included two predictors. Results indicate that students who recently lived on a farm were 5.9 times as likely to seroconvert, and those who completed two elective courses were 9.3 times as likely (Table 5).

Random forest

With a total of 13 cases, the random forest algorithm correctly predicted seroconversion 30.7% of the time with a sensitivity of 30.8% and specificity of 99.0%. Variables with the greatest predictive value were, respectively, the number of elective courses taken, recent contact with goats, recent contact with sheep, gender, and having recently lived on a farm (Figure 2).

DISCUSSION

During the four-year study period, 19.4% (13/67) of veterinary students seroconverted, a figure similar to the 17% incidence of seroconversion found in a study of Dutch cullers, an extremely high-risk group (Whelan, 2011). Yet unlike the cullers, the lack of awareness regarding infection status among seropositive students suggests all cases were mild or asymptomatic. Assuming veterinary students are generally subject to chronic, low-intensity exposures, these findings support the existing hypothesis that inoculum size determines the severity of acute Q fever (van der Hoek, 2012).

We chose to analyze the data by both the less familiar random forest method in addition to classical logistic regression due to underlying assumptions of generalized linear models that may not be most appropriate in this case. Notably, both the regression and random forest models identified "elective courses" as the top predictor for seroconversion. The final multivariate regression model included "recently living on a farm" as the only other statistically significant predictor. In the random forest model, goat contact, sheep contact and gender were among the top predictors and of greater importance than recently living on a farm. Differences in the final models could possibly be due to the limited sample size and moderate, but influential, correlations between predictors. The true risk of recently living on a farm can be largely attributed to goat and sheep contact, variables that showed a clear association with seroconversion at one of the follow-up periods. Risk from the farm environment itself is the least important predictor in random forest. Similarly, 33% of males seroconverted in 2008, supporting the role of gender as a risk factor though the p-value is insignificant at $p<0.05$.

Environmental exposure to *C. burnetii* cannot be ignored as a potential confounder given the epidemic's peak in 2009. The lack of general population seroprevalence data in the Utrecht area after 2006 leaves little indication of the extent to which the epidemic may have increased background rates. Utrecht was outside the epicenter, though infections in the area were reported. Nevertheless, no evidence of environmental transmission was found during analysis – the variable "year" was highly insignificant, and the seroconversion rates before and after the epidemic peak are comparable. Since all students began VMS in Utrecht at the same time, differences in their environmental exposures while away from university is the greatest potential concern. Without permanent addresses, we could not capture the exposures of students who spent time in Q fever-affected areas unless they "lived on a farm" during those periods.

Model performance was limited by substantial challenges, yet random forest correctly predicted over a third of seroconverted students. Male gender, goat contact, sheep contact and/or living on a farm positively predicted infection. Specific associations between academic curriculum and seroconversion have not been reported before but are biologically plausible and provide insight into potential opportunities for public health intervention. Vaccines are rarely offered due to the added diagnostic requirement of pre-screening for a previous *C. burnetii* infection to avoid potential adverse effects. In the case of veterinary students, vaccination may provide an opportunity for intervention especially for students pursuing livestock medicine or with underlying medical conditions increasing the risk posed by a potential Q fever infection. Even more, awareness programs such as promoting periodic testing for asymptomatic infections may be beneficial and encourage proactive screening during the students' future careers. In all likelihood, Q fever is a greater health burden for veterinary students than previously realized and future work is needed to elucidate the implications.

Table 1: Characteristics of all students who completed baseline questionnaires and serology, N(%)*

Variable	Seronegative N=100	Seropositive N=13	p-value[1]
Gender			0.480
Male	21 (21.0)	4 (30.8)	
Female	79 (79.0)	9 (69.2)	
Smoker			0.613
Never	92 (92.0)	13 (100.0)	
Former	3 (3.0)	0 (0)	
Current	4 (4.0)	0 (0)	
Prior VMS[2] at other university			--
None	93 (93.0)	13 (100.0)	
<1 year	1 (1.0)	0 (0)	
1 year	4 (4.0)	0 (0)	
2 years	2 (2.0)	0 (0)	
Feel healthy			1.0
No	2 (2 .0)	0 (0)	
Yes	97 (97.0)	13 (100.0)	
Zoonosis within past year			0.414
No	85 (85.0)	10 (76.9)	
Yes	14 (14.0)	3 (23.1)	
No. Years Lived on a Farm			<0.001
None	87 (87.0)	4 (30.8)	
1-9	7 (7.0)	2 (15.4)	
10+	6 (6.0)	7 (53.8)	
Ever had reg. sheep contact			0.135
No	82 (82.0)	8 (61.5)	
Yes	18 (18.0)	5 (38.5)	
Ever had reg. bovine contact			<.001
No	82 (82.0)	3 (23.1)	
Yes	18 (18.0)	10 (76.9)	
Ever had reg. goat contact			0.002
No	86 (86.0)	6 (46.1)	
Yes	14 (14.0)	7 (53.9)	
Ever had reg. dog contact			0.537
No	23 (23.0)	4 (30.8)	
Yes	77 (77.0)	9 (69.2)	
Ever had reg. cat contact			0.344
No	30 (30.0)	2 (15.4)	
Yes	70 (70.0)	11 (84.6)	

[1] Pearson's chi-square or Fisher's Exact

[2] Veterinary Medical School

* May not sum to total due to missing values

Table 2: Baseline characteristics of students included in study compared to those lost to follow-up, N(%)*

Characteristic	Study Population (N=67)	Lost to Follow-up (N=33)
Age (years), mean ± SD	19.5 ± 1.9[1]	20.3 ± 2.3[1]
Gender, n (%)		
Male	10 (14.9)	11 (33.3)
Female	57 (85.1)	22 (66.7)
Active Smoker, n(%)		
No	65 (97.0)	28 (84.9)
Yes	2 (3.0)	3 (9.1)
Ever Lived on Farm		
No	59 (88.1)	28 (84.9)
Yes	8 (11.9)	5 (15.1)
# Years Lived on Farm		
None	59 (88.1)	28 (84.9)
<10	3 (4.4)	4 (12.1)
≥10	5 (7.5)	1 (3.0)
Ever Reg. Sheep Contact		
No	53 (79.1)	29 (87.9)
Yes	14 (20.9)	4 (12.1)
Ever Reg. Bovine Contact		
No	54 (80.6)	28 (84.9)
Yes	13 (19.4)	5 (15.1)
Ever Reg. Goat Contact		
No	57 (85.1)	29 (87.9)
Yes	10 (14.9)	4 (12.1)
Ever Reg. Cat/Dog Contact		
No	4 (6.0)	4 (12.1)
Yes	63 (94.0)	29 (87.9)

[1] Mean +/- standard deviation

*May not sum to total due to missing values

Table 3: Exposures frequencies and Q fever seroconversion, by follow-up year, N(%)*

	2008 Follow-up		2010 Follow-up	
Predictor	**n (%)**	**Seroconversion n(%)**	**n(%)**	**Seroconversion n(%)**
	n = 61 (100.0)	8 (13.1)	n = 49 (100.0)	5 (10.2)
Age				
<19	30 (49.2)	4 (13.3)	0 (0)	0 (0)
≥19	31 (50.8)	4 (12.9)	49 (100.0)	5 (10.2)
Gender				
Male	9 (14.8)	3 (33.3)	5 (10.2)	0 (0)
Female	52 (85.2)	5 (9.6)	44 (89.8)	5 (11.4)
Active smoker				
No	59 (96.7)	8 (13.6)	48 (98.0)	5 (10.4)
Yes	2 (3.3)	0 (0)	1 (2.0)	0 (0)
Primary housing				
Student house	35 (57.4)	4 (11.4)	24 (49.0)	3 (12.5)
Private house	9 (14.8)	0 (0)	15 (30.6)	2 (13.3)
Parental house	9 (14.8)	1 (11.1)	7 (14.3)	0 (0)
Other	8 (13.1)	3 (37.5)	3 (6.1)	0 (0)
# years lived on farm				
None	53 (86.9)	5 (9.4)	45 (91.8)	5 (11.1)
<10	3 (4.9)	0 (0)	2 (4.1)	0 (0)
≥10	5 (8.2)	3 (60.0)	2 (4.1)	0 (0)
Recently lived on farm				
No	51 (83.6)	4 (7.8)	44 (89.8)	4 (9.1)
Yes	10 (16.4)	4 (40.0)	5 (10.2)	1 (20.0)
Zoonosis since start VMS				
No	56 (91.8)	8 (14.3)	40 (81.6)	4 (10.0)
Yes	5 (8.2)	0 (0)	9 (18.4)	1 (11.1)
Completed pre/postnatal sheep & lambing elective				
No	28 (47.5)	2 (7.1)	21 (46.7)	0 (0)
Yes	31 (52.5)	6 (19.4)	24 (53.3)	4 (16.7)
Completed pain management elective				
No	34 (60.7)	3 (8.8)	30 (63.8)	3 (10.0)
Yes	22 (39.3)	5 (22.7)	17 (36.2)	2 (11.8)
No. elective courses completed				
None	18 (29.5)	1 (5.6)	15 (30.6)	1 (6.7)
One	33 (54.1)	3 (9.1)	27 (55.1)	2 (7.4)
Two	10 (16.4)	4 (40.0)	7 (14.3)	2 (28.6)
Recent contact with goats				
No	57 (93.4)	8 (14.0)	45 (91.8)	4 (8.9)
Yes	4 (6.6)	0 (0)	4 (8.2)	1 (25.0)
Recent contact with sheep				
No	51 (83.6)	4 (7.8)	45 (91.8)	5 (10.4)
Yes	10 (16.4)	4 (40.0)	4 (8.2)	0 (0)
Recent contact with bovine				
No	57 (93.4)	7 (12.3)	45 (91.8)	5 (11.1)
Yes	4 (6.6)	1 (25.0)	4 (8.2)	0 (0)
Recent contact with domestic animals				
No	9 (14.8)	2 (25.0)	7 (14.3)	1 (14.3)
Yes	52 (85.3)	6 (75.0)	42 (85.7)	4 (9.5)

Table 4: Bivariate Analysis of Factors Associated with Seroconversion (OR (95% Confidence Interval))

Predictor	OR (95% CI)	p-value
Age		0.763
<19	1.0	
≥19	0.82 (0.23, 2.91)	
Gender		0.244
Male	1.0	
Female	0.43 (0.10, 1.8)	
Zoonosis since start VMS		0.568
No	1.0	
Yes	0.538 (0.07, 4.50)	
Recently lived on farm		0.010
No	1.0	
Yes	5.44 (1.49, 19.85)	
# years lived on farm		0.077
None	1.0	
<10	--	
≥10	6.60 (1.29, 33.81)	
# electives completed		0.012
None	1.0	
1	1.41 (0.26, 7.70)	
2	8.45 (1.48, 48.25)	
Recent sheep contact		0.007
No	1.0	
Yes	6.11 (1.65, 22.67)	
Recent bovine contact		0.951
No	1.0	
Yes	1.07 (0.12, 9.48)	
Recent goat contact		0.951
No	1.0	
Yes	1.07 (0.12, 9.48)	
Recent domestic animal contact		0.360
No	1.0	
Yes	0.52 (0.13, 2.21)	

Table 5: Multivariate Analysis of Factors Associated with Seroconversion, Generalized Linear Mixed Model, p<0.3 (OR, (95% Confidence Interval))

Predictor	OR (95% CI)	p-value
Recently lived on farm		0.028
No	1.0	
Yes	5.87 (1.32, 26.17)	
No. electives completed		
None	1.0	
One	1.86 (0.29, 11.87)	0.507
Two	9.28 (1.37, 62.99)	0.023

Figure 1: Study Timeline

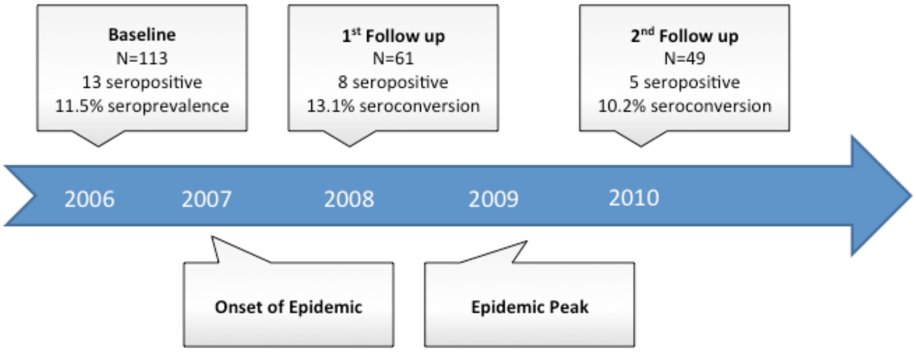
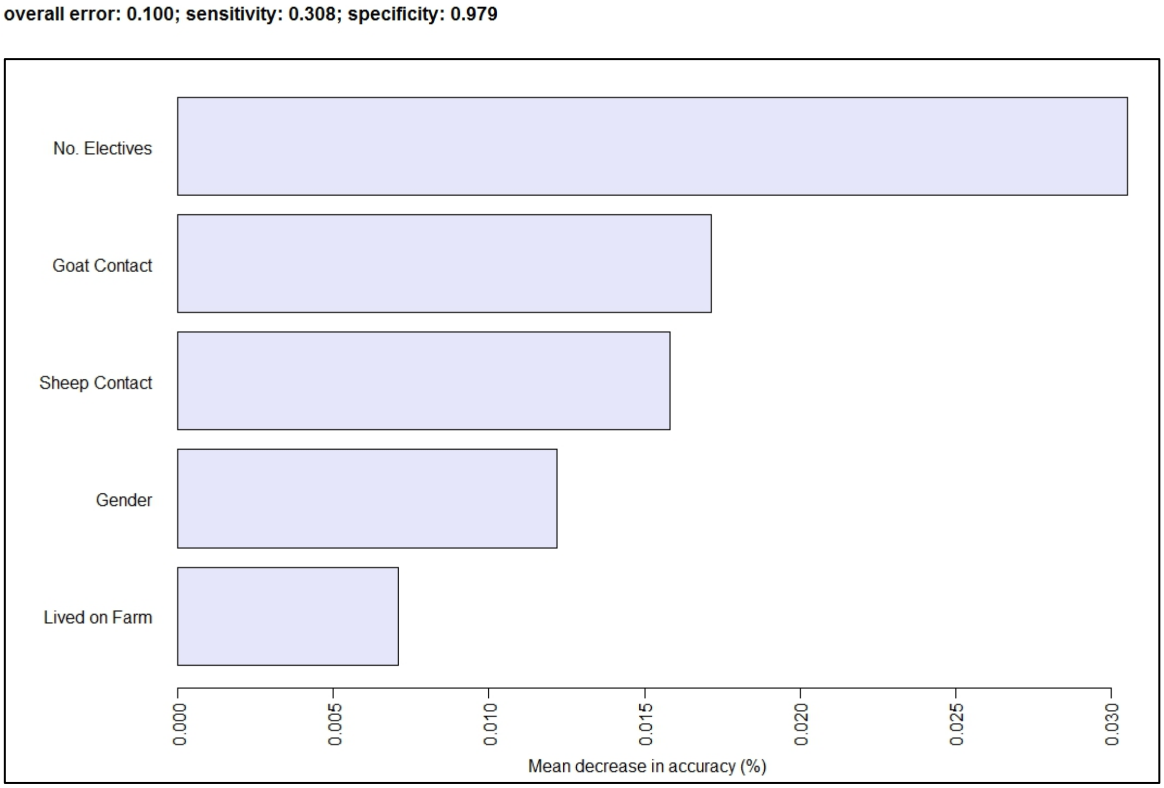


Figure 2: Random forest model of top predictors for seroconversion



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