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Epizootiological investigation of a Q fever outbreak and implications for future control strategies

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Objective—To describe the epizootiological investigation of an outbreak of Q fever (*Coxiella burnetii* infection).

Design—Epidemiological study.

Animals—17 goat herds in Washington, Montana, and Oregon.

Procedures—In April 2011, an abortion storm at a commercial goat farm in Washington was determined to be caused by *C burnetii*. A joint epidemiological investigation by public health and veterinary professionals was subsequently performed to assess the extent of the outbreak by performing a trace-forward of goats sold from the index farm, to determine risk factors associated with infection, and to implement control measures. A herd management plan was developed to control the outbreak and reduce risk of human exposure. Quarantine and temporary holds preventing the sale or movement of goats allowed time for trace-forward investigation, education of farmers regarding disease risk, and testing to determine the scope of the outbreak.

Results—17 farms were affected; 21 human Q fever cases were identified. Bacterial shedding in feces, vaginal fluid, or milk was confirmed in 156 of 629 (25%) goats tested by PCR assay. Seroprevalence of antibodies against *C burnetii* in goats, determined by ELISA, was 12%. The risk for *C burnetii* infection in goats was highest among females, those on farms associated with human Q fever, and those on Washington farms. A protective effect was observed for goats at farms where the primary form of goat carcass disposal was burial.

Conclusions and Clinical Relevance—This outbreak illustrated the importance of a joint investigation for zoonotic pathogens and the need to expand and strengthen relationships between medical, public health, and veterinary partners. Heightened awareness and enhanced veterinary diagnostic capabilities for *C burnetii* are needed to identify and control outbreaks expediently. (*J Am Vet Med Assoc* 2015;247:1379–1386)

Q fever is a zoonotic disease caused by the bacterial pathogen *Coxiella burnetii*, which can affect many animal species worldwide.[1] Cattle, sheep, and goats are considered the primary reservoirs. Infected animals excrete the organisms in the highest load at parturition in birth products, but also in feces, urine, milk, sputum, semen, and vaginal fluids.[2,3] Transmission to humans and other animals most commonly occurs by contact with animal birth products or by inhalation of pathogen-contaminated dust or aerosols.[1] Transmission might also occur via tick bite or ingestion of contaminated milk.[4,5] *Coxiella burnetii* is a highly infectious

Abbreviation

CI	Confidence interval

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pathogen with an estimated 50% infectious dose in humans of 1.18 bacteria.[6]

Most infections in animals caused by *C burnetii* (termed coxiellosis) are subclinical. If clinical illness occurs in ruminants, it primarily involves reproductive disorders such as abortion, stillbirth, endometritis, mastitis, and infertility.[7] Large outbreaks in people have been associated with abortion storms in infected animal herds. A Q fever outbreak associated with dairy goat farms in The Netherlands from 2007 through early 2010 resulted in over 4,000 reported cases of human infection.[8] Illness occurs in approximately half of the individuals infected, and the most common manifestation of acute disease is a flu-like illness, with pneumonia and hepatitis occurring in more severe cases.[1] Unlike other animals, humans can also develop chronic Q fever that typically results in endocarditis or vascular infection in those with preexisting risk factors such as valvular or vascular defects.[1]

Diagnosis of Q fever in humans is most commonly made on the basis of antibody detection through serologic testing.[9] In animals, serologic testing has not been shown to be a reliable method to determine whether infected animals are a potential source of transmission to humans or other animals. A positive antibody titer result in an infected animal does not correspond to shedding of organisms, and some seronegative animals may still actively shed bacteria.[10–14] Quantitative PCR assay of biological matter or fluids (eg, feces, milk, or vaginal fluid) is a rapid and highly sensitive method of diagnostic testing for *C burnetii* infection.[15,16] It has the advantage of determining the number of animals in a herd currently shedding the organism and quantifying the bacterial load.

In April 2011, an abortion storm at a Boer goat farm used for commercial meat, show, and breeding stock in Washington (farm 1) was determined to be caused by *C burnetii* infection. Fourteen of 50 (28%) pregnant does in the herd had aborted during January through March of that year. In May 2011, a Washington resident who had purchased goats from farm 1 was diagnosed as having Q fever. Ultimately, 21 cases of the disease were identified in Montana (9 cases) and Washington (12 cases) among persons who had contact with goats purchased from farm 1. Detailed descriptions of the human cases and the related epidemiological investigation have been published elsewhere.[17,18] The epizootiological investigation reported here details the laboratory results and trace-forward investigation of the farm 1 (index) goat herd, specifies animal-testing strategies used, and describes recommended herd control measures to decrease disease transmission to both domestic animals and humans.

During the abortion storm at farm 1, the farm owners and veterinarian made multiple attempts to obtain a diagnosis, but the etiology remained unknown. On March 29, 2011, the placenta from a female goat that had given birth to 2 stillborn fetuses was submitted to a veterinary diagnostic laboratory. On April 22, 2011, *C burnetii* was detected by immunohistochemical analysis of the placental specimen. Testing for *C burnetii* was not initially performed by the laboratory, as it was not part of routine diagnostic testing of tissues in cases of small ruminant abortion. After only negative results from tests for other potential etiologies and urging from the farm owner and their veterinarian to identify the cause of the outbreak, the laboratory included additional immunohistochemical testing for *C burnetii*.

This was the first abortion storm to occur on farm 1, although it had been in operation since 2003. The source of the infection for the farm was unknown, but multiple opportunities existed for farm 1 goats to be exposed to the pathogen through show and breeding activities conducted by the farm throughout Washington and in other states.

Materials and Methods

Trace-forward activities—Immediately after *C burnetii* was detected by immunohistochemical analysis of the placental specimen from farm 1 in April, a report was made to the Washington State Department of Agriculture, then via the Washington State Department of Health to the Grant County Health District. Consequently, the farm owners and public health officials began to notify recent goat purchasers that *C burnetii* infection had been detected in the farm 1 goat herd. These individuals were informed of the possibility that goats purchased from farm 1 might be infected. Farm 1 owners provided sales records to the outbreak investigation team that listed available contact information of persons who had purchased goats from their farm in the preceding year. Contact information was available only for persons who purchased live animals.

Outbreak control measures—A statewide health alert notice in Washington and county health alert notices for affected communities in Washington and other states identified during the investigation were released in order to raise physician awareness of Q fever. Following confirmation of the first identified human case of Q fever associated with this outbreak (in Washington during May 2011), the index farm and a second farm (farm 2) in Washington that had purchased goats from farm 1 and was also experiencing goat abortions were placed under quarantine by the state veterinarian. All other Washington farms identified through the investigation were placed under a temporary hold in which farmers were requested to voluntarily cease all movement of goats from the herd. Farms in other states identified as a potential risk to human health by the state veterinarian were also placed under a temporary hold.

A herd management plan[a] recommending best practices to prevent and control animal infection, and to encourage collaboration between public health and animal health officials, was developed and distributed to all affected farms during the outbreak investigation. The herd management plan was modeled on the Montana Department of Livestock official herd plan for brucellosis and revised for each farm as needed, in partnership with the office of the State Veterinarian. Key components of the plan included education, detailed animal record keeping, control and testing strategies, and notification requirements for public health and veterinary authorities.

Animal testing for coxiellosis—A goat herd was included for coxiellosis testing if the farm was identi-

fied through farm 1 sales records as purchasing ≥ 1 goat from June 2010 through June 2011 and if the farm owner agreed to participate. Although the abortion storm at farm 1 began in January 2011, the trace-forward included goats sold since June 2010. This decision was made in an effort to detect potential human cases infected via exposure to goats that had subclinical disease or were incubating the bacteria prior to farm 1's abortion storm. To maximize the probability of capturing the true seroprevalence and extent of bacterial shedding in each herd, the sampling goal for each herd was 100% whenever possible. Samples of blood, vaginal secretions, milk, or feces (or multiple sample types) were collected from included animals in June 2011. Farmers were surveyed at the same time as animal sampling. A written questionnaire focused on history of illness in the family or among visitors, farm management techniques (eg, disinfection practices and disposal of manure and carcasses), and history of animal illnesses, including adverse pregnancy events, in the herd. For this investigation, an adverse pregnancy event was defined as any abortion, stillbirth, or birth of weak neonates that had occurred in the herd within the previous 12 months.

Laboratory analysis—Samples of blood, vaginal fluid, milk, and feces were collected from goats on participating farms for professional laboratory analysis, although not all goats had all specimen types collected for testing. Vaginal fluid samples were obtained from the vaginal mucosa with a dry, sterile culture swab. Blood samples were obtained from a jugular vein and collected in 5-mL serum separator tubes. Individual milk samples were obtained by manual milking into sterile, 50-mL plastic tubes without preservatives. Fecal samples were collected per rectum with a sterile culture swab.

Serum samples from goats were tested for antibodies against *C burnetii* by means of ELISA. A commercial ELISA kit[b] employing the phase I-II Nine Mile antigen strain of *C burnetii* was used according to manufacturer's instructions and by methods previously described.[19]

Vaginal and fecal swab samples were processed for PCR assay by placing the swab in a 2-mL tube with 800 µL of sterile PBS solution, vortexing briefly, and then incubating at room temperature (approx 15° to 25°C) for 1 hour with gentle rotation. After incubation, 200 µL was removed from the tube for DNA extraction. For vaginal swabs, DNA was extracted according to the kit manufacturer's protocol for tissue.[c] For fecal swabs, DNA was extracted according to the manufacturer's protocol for processing of fecal samples.[c] Milk collected from goats was stored at 4°C. The milk was centrifuged for 15 minutes at 1,700 × g, washed with PBS solution (pH, 7.4), and then centrifuged again for 15 minutes at 1,700 × g. The pellets were resuspended in 1 mL of PBS solution and then centrifuged at 20,000 × g for 5 minutes. The pellets were resuspended in 200 µL of manufacturer-supplied optimized tissue lysis buffer, and DNA was purified with the tissue protocol from the same kit.[c] For these protocols, DNA was eluted in 200 µL of a tris-EDTA buffer supplied with the kit. Quantitative PCR assay was performed on 1 µL of the eluted DNA in a 25-µL reaction volume. Primers targeting the IS1111a insertion sequence were used to detect *C burnetii* according to published procedures.[20] Samples with a crossing threshold value < 40 were considered positive. If any biological sample from an animal tested positive by PCR assay, the animal was identified as a *C burnetii* shedder.

Statistical analysis—A statistical software package was used for the analysis and comparison of infection status among goats.[d] The outcome of interest was *C burnetii* infection. Any goat with test results classified as suspect (according to the manufacturer's instructions) on the ELISA test was removed from analysis. Variables found to be associated on initial univariate analysis by means of a log-likelihood χ^2 test were considered for further multivariable logistic modeling. Odds ratios were used to measure associations between *C burnetii* infection in goats and exposures of interest. Ninety-five percent CIs were calculated where appropriate by use of an exact binomial method. Goodness of fit was determined by calculating the Hosmer-Lemeshow goodness-of-fit χ^2 statistic. Values of $P < 0.05$ were considered significant in all statistical tests on the basis of the likelihood ratio χ^2 statistic. Factors associated with infection were further evaluated with multivariable logistic modeling by means of a backward stepwise elimination technique.

Sensitivity and specificity of serologic testing with PCR assay results used as the standard reference were calculated by use of standard epidemiological methods.[21] Sample size was calculated for estimating detection of *C burnetii* infection in goats via PCR assay and serologic testing, assuming an average estimated disease prevalence of 10%, a 5% probability of incorrectly concluding that a healthy population is diseased, and a CI of 95%, and was derived from calculations to determine sample size with imperfect tests as described previously.[22]

Results

Trace-forward activities—Goats sold by farm 1 from June 2010 to June 2011 were traced to 21 farms in Washington, Montana, and Oregon. All the farms were contacted; owners were provided with contact information for obtaining answers to veterinary or public health questions and were given an informational document with frequently asked questions and answers regarding Q fever. Seventeen farms participated in the epizootiological investigation (13 in Washington, 3 in Montana, and 1 in Oregon). None of the farmers who purchased goats from farm 1 reported selling those goats onward to other farms. Twenty-one human Q fever cases were identified that were associated with 4 farms in Washington and 2 farms in Montana.

Control measures—The first human Q fever case identified, which initiated the joint epidemiological investigation, was reported by a physician in May 2011; this physician had read the county health alert regarding Q fever risk in the community owing to the disease outbreak in goats. The quarantines and temporary holds placed on farms were lifted at the conclusion of the investigation when the affected farms instituted the herd management plan[a] in partnership with their state veterinarian's office. No new human cases of the disease associated with this outbreak were identified in the affected states after August 2011.

Animal testing for coxiellosis—A total of 567 goats from 17 farms were tested by ELISA for the presence of serum antibodies against *C burnetii* (**Table 1**). The median number of goats tested per herd was 24 (range, 5 to 133 goats). Results were positive for 70 goats; 9 goats had ELISA results considered suspect for the disease. The overall seroprevalence of antibodies against *C burnetii* in goats was 12% (70/567). There was no significant difference in seropositivity between male and female goats. Overall, 10 of 17 herds tested had ≥ 1 seropositive goat identified. There was significant ($P < 0.001$) variation in the proportion of goats that were seropositive among the farms.

Of 629 goats that had ≥ 1 specimen tested for *C burnetii* shedding by PCR assay, 156 (25%) had a positive result. Vaginal fluid samples were collected from 449 goats; of these, 131 (29%) had a positive PCR assay result. Shedding was detected in fecal samples from 22 of 192 (11%) goats and in milk samples from 28 of 70 (40%) goats (**Figure 1**). At the herd level, 14 of 17 herds tested positive for *C burnetii* shedding in ≥ 1 specimen.

Of the 87 goats that underwent serologic testing and were identified as shedding *C burnetii* in ≥ 1 biological sample type (vaginal fluid, milk, or feces) by PCR assay, only 23 (26%) had a positive anti–*C burnetii* antibody test result. Goats that were serologically tested and were categorized as heavy shedders (defined as shedding > 1,000 genome equivalents/sample in ≥ 1 biological sample type by PCR assay) totaled 39; 16 (41%) of these goats had a positive antibody test result.

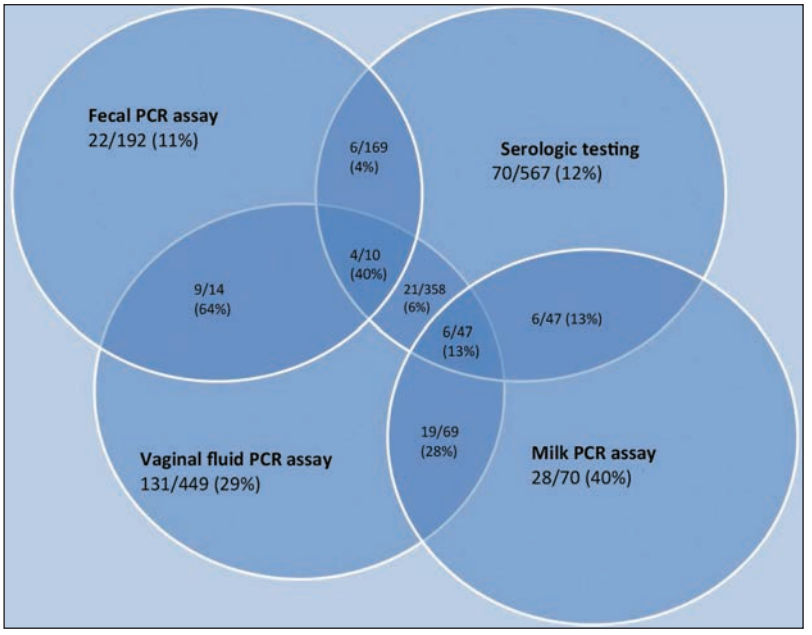

Figure 1—Venn diagram of serologic (ELISA) and PCR assay results for the presence of antibodies against *Coxiella burnetii* and presence of *C burnetii* DNA, respectively, in biological samples from goats of 17 herds in Washington, Montana, and Oregon tested as part of an epizootiological investigation of an outbreak of Q fever in 2011. The overlapping areas in the diagram illustrate the proportion of animals that had positive results for each of the overlapping specimen-test types (eg, of 47 goats that underwent serologic testing, PCR analysis of vaginal fluid, and PCR analysis of a milk sample, 6 [13%] had positive results in all 3 specimen types).

Table 1—Farm-level seroprevalence of antibodies against *Coxiella burnetii* and PCR detection of the organism in samples collected from goats of 17 herds in Washington, Montana, and Oregon during a 2011 epizootiological investigation of an outbreak of Q fever.

Farm	Proportion (%) of animals seropositive*	Proportion (%) of samples positive by PCR assay†			Reported APE in goat herd	Associated human cases
		Vaginal fluid	Milk	Feces		
Washington						
1	12/58 (21)	72/113 (64)	19/21 (90)	5/42 (12)	Yes	4
2	2/22 (9)	0/15 (0)	0/3 (0)	0/5 (0)	Yes	5
3	1/34 (3)	5/36 (14)	0/11 (0)	—	Yes	0
4	7/15 (47)	1/10 (10)	—	0/6 (0)	Yes	2
5	5/20 (25)	6/11 (55)	—	2/7 (29)	Yes	0
6	1/24 (4)	3/19 (16)	0/2 (0)	0/5 (0)	Yes	0
7	1/30 (3)	0/19 (0)	—	0/12 (0)	No	0
8	0/5 (0)	2/3 (67)	—	0/2 (0)	No	0
9	0/49 (0)	0/41 (0)	—	2/6 (33)	Yes	1
10	7/9 (78)	0/7 (0)	—	0/2 (0)	No	0
11	0/14 (0)	6/12 (50)	—	0/3 (0)	Yes	0
12	0/15 (0)	3/11 (27)	—	1/4 (25)	No	0
13	0/29 (0)	1/16 (6)	—	1/14 (7)	No	0
Montana						
14	1/50 (2)	4/28 (14)	0/2 (0)	0/7 (0)	Yes	2
15	33/55 (60)	25/27 (93)	8/8 (100)	11/15 (73)	Yes	7
16	0/133 (0)	2/76 (3)	1/23 (4)	0/58 (0)	Yes	0
Oregon						
17	0/5 (0)	1/5 (20)	—	0/4 (0)	No	0
Total	**70/567 (12)**	**131/449 (29)**	**28/70 (40)**	**22/192 (11)**	—	**21**

Farm 1 was the index farm. In the epizootiological investigation, goats were traced from farm 1 to 21 farms in Washington, Montana, and Oregon. Participation in the investigation was voluntary; 17 of 21 farms were included. All proportions are based on the number of animals that had the test performed. Not all animals had samples of every type collected.

*Serum samples from goats were tested for antibodies against *C burnetii* by means of an ELISA with the phase I-II Nine Mile antigen strain of *C burnetii*. †The PCR assay was performed on purified DNA by use of primers targeting the *C burnetii* IS111a insertion sequence.

— = Not applicable. APE = Adverse pregnancy event (1 or more in 12 months prior to the time of sample collection on the farm, including abortions, stillbirths, or births of weak neonates).

Twenty-five of 40 (63%) shedding goats that had > 1 sample type tested were found to shed the organism by > 1 route. Goats shedding *C burnetii* in any specimen were 4 times as likely to be seropositive for anti–*C burnetii* antibodies, compared with goats in which shedding was not detected. Sixty-four of 87 (74%) goats that tested positive for shedding the organism in ≥ 1 sample type and also underwent serologic testing had a negative antibody result; 388 of 422 (92%) goats that underwent serologic testing and had testing of ≥ 1 sample type by PCR assay, but were not shedding the organism, were seronegative for these antibodies.

There was a statistically significant association between seropositivity for antibodies against *C burnetii* and shedding of the organism in goats (OR, 4.1; 95% CI, 2.3 to 7.4). The strongest predictor of seropositivity in goats that shed *C burnetii* was shedding the organism in milk; these goats were 74 times as likely (95% CI, 6.6 to 833.9) to test positive for anti–*C burnetii* antibodies as goats that shed the organism in other biological fluids or matter. Goats that shed *C burnetii* in feces or vaginal fluid were 3.4 times (95% CI, 1.2 to 10.3) and 5.1 times (95% CI, 2.6 to 10.0) as likely to have a positive serologic test result, respectively, as were goats that shed the organism in other biological fluids or matter.

The sensitivity and specificity of serologic testing for *C burnetii* infection were calculated to be 26% and 92%, respectively, with PCR assay results used as the standard reference. Sample size calculations that can be used for serologic and PCR testing in goat herds assuming an average estimated disease prevalence of 10%, a 5% probability of incorrectly concluding that a healthy population is diseased, and a CI of 95% were provided **(Table 2)**.

Risk factor analysis—For purposes of risk factor analysis, goats were identified as infected if they tested positive for the presence of anti–*C burnetti* antibodies in serum by ELISA or had a positive PCR assay result for *C burnetii* shedding in ≥ 1 biological sample type. A total of 674 goats on 17 farms underwent testing of ≥ 1 type of specimen. Of these, 169 (25%) were infected with *C burnetii*. On a herd level, 17 of 17 farms had ≥ 1 infected goat identified.

In the univariate risk analysis, 6 factors were significantly associated with *C burnetii* infection in goats: lactation, female sex, residence at a Washington farm location, residence at a farm associated with human Q fever, residence at a farm that reported ≥ 1 adverse pregnancy event in the herd within the previous 12 months, and type of carcass disposal. Other risk factors examined, including use of disinfection methods in the birthing area after kidding, manure disposal methods, and herd size, were not associated with infection. In the final multivariable logistic regression model, 4 factors were significantly associated with *C burnetii* infection status in goats **(Table 3)**; the strongest predictor was residence at a farm associated with human Q fever, followed by residence at a farm in the state of Washington. Female goats were more than twice as likely to be infected with *C burnetii* as were males. A protective effect was observed for goats residing at farms where burial was the primary form of carcass disposal on death of a goat (compared with composting, incinerating, or using a local landfill site).

Table 2—Herd sample sizes required for detection of *C burnetii* infection in goat herds via PCR assay and serologic testing assuming an estimated disease prevalence of 10%, 5% probability of incorrectly concluding a healthy population is diseased, and 95% CI.*

Herd size	Sample size required for PCR assay†	Sample size required for serologic testing‡
30	30	30
50	39	50
100	55	98
150	58	145
200	60	191
300	62	280
500	63	447
1,000	63	808

Calculations were based on sensitivity and specificity of serologic testing for *C burnetii* infection, with PCR assay results used as the standard reference from data analyzed in this outbreak investigation.

*Regardless of test type, specimens should be collected first from those animals at highest risk of infection, including goats that have recently had an adverse pregnancy event, goats that have recently kidded, pregnant goats, and lactating goats. †Milk specimens are preferred for lactating animals; otherwise, vaginal swab specimens can be used. Male goats can be tested by analysis of fecal samples. ‡If required sample size testing is not feasible, serologic testing of the number of animals calculated for testing by PCR assay can be used, with targeting of high-risk animals for testing; this will increase the probability of identifying infected animals but will not provide an estimate of *C burnetii* infection prevalence for the herd and may result in a herd being incorrectly classified as infection free.

Table 3—Results of multivariable logistic regression for risk of *C burnetii* infection among goats on 17 farms in Washington, Montana, and Oregon, 2011.

Variable	OR (95% CI)	*P* value
Sex		
Female	2.8 (1.8–4.5)	< 0.001
Male	Referent	—
State		
Washington	3.1 (2.0–4.7)	< 0.001
Oregon	0.9 (0.1–8.2)	0.913
Montana	Referent	—
Farm associated with human Q fever		
Yes	5.5 (3.7–8.3)	< 0.001
No	Referent	—
Type of carcass disposal		
Burial	0.1 (0.1–0.2)	< 0.001
Incineration	1.4 (0.7–2.8)	0.280
Compost	1.3 (0.8–1.9)	0.494
Landfill	Referent	—
Lactating		
Yes	1.8 (0.9–3.3)	0.054
No	Referent	—
Adverse pregnancy event in herd within last 12 mo		
Yes	0.7 (0.3–1.4)	0.105
No	Referent	—

For purposes of this analysis, goats were considered infected if they tested positive for the presence of anti–*C burnetii* antibodies in serum by ELISA or had a positive PCR assay result for *C burnetii* shedding in ≥ 1 biological sample type. Values of $P < 0.05$ were considered significant.

— = Not applicable.

Discussion

Goats are a common source of human infection with *C burnetii*, the causative agent of Q fever, globally.[23] In the United States, researchers have reported that goats have the highest estimated species-specific prevalence of antibodies against *C burnetii*, at up to 42%.[24] The overall seroprevalence of antibodies against this pathogen in goats of the present study, 70 of 567 (12%), was similar to results from a study[25] conducted by the Washington Animal Disease Diagnostic Laboratory in 2010 and 2011, in which 1,794 serum specimens from goats in Washington submitted for routine serologic screening were tested for the presence of anti–*C burnetii* antibodies. In that study, 144 (8%) samples had positive test results. Although the USDA's National Animal Health Monitoring System collects nationally representative data for livestock diseases, sampling of goats for *C burnetii* infection has not been performed at that level, and accurate estimates of regional differences in infection are not available. A larger, randomized, and more representative study is needed to estimate the geographic distribution of *C burnetii* infection among goats in the northwestern United States and in the country as a whole.

Female sex and residence at farms associated with human cases of Q fever were biologically logical risk factors found to be significantly associated with *C burnetii* infection in goats of the present study. The bacterium localizes predominantly in the female reproductive tract, and long-term shedding through vaginal secretions or mammary glands has been demonstrated.[3,12,26] The protective effect found for burial as the primary means of carcass disposal (where the odds of *C burnetii* infection in goats were approx one-tenth of those for goats on farms where carcasses were disposed of in local landfills) might reflect better prevention against scavenging, which was frequently reported by farmers that used other methods of carcass disposal. Burial is often a preferred method of animal disposal during infectious outbreaks because it prevents transport of carcasses off-site, which might pose a public health risk. Improper burial, however, may pose a threat to groundwater quality and lead to environmental contamination.[27,28] Carcass disposal at a public landfill might also be a viable alternative for *C burnetii*–infected carcasses. Appropriately engineered landfill sites pose a low risk for animal or human disease transmission, and transport can be performed quickly after animal death.[27,29] Local and state regulation on use of landfill sites for carcass disposal varies.

In our investigation, milk samples tested positive for shedding of *C burnetii* more frequently than did feces or vaginal fluids (28/70 [40%] vs 22/192 [11%] and 131/449 [29%], respectively). Concomitant shedding was common; 25 of 40 (63%) shedding goats that had > 1 sample type tested were found to shed the organism by > 1 route. Goats shedding *C burnetii* in any specimen were 4 times as likely to be seropositive for anti–*C burnetii* antibodies, compared with goats in which shedding was not detected. Goats that are shedding the organism pose the highest risk for transmission to people; however, 64 of 87 (74%) goats that tested positive for shedding the organism in ≥ 1 sample type and also underwent serologic testing had a negative antibody result. Additionally, most (388/422 [92%]) of the goats that underwent serologic testing and testing of ≥ 1 sample type by PCR assay, but were not shedding the organism, were seronegative for these antibodies. Although the most common method to evaluate a herd for infection is serologic testing, it is clear that this method alone can result in a gross underestimation of a herd's prevalence of infection or even a false-negative herd classification.

Clinical data on individual goats were not available during this investigation, so it was not possible to evaluate an association between infection status and adverse pregnancy events. However, only the index farm experienced an abortion storm. At least 1 adverse pregnancy event in a doe was reported for 11 of the 17 farms during the 12 months prior to the investigation. There are multiple causes of abortion or stillbirth in a goat herd; some losses are expected, and not all warrant investigation. Losses in a goat herd severe enough to seek diagnosis have been defined as abortions in ≥ 5% of pregnant animals in large herds (> 100 animals) or in > 3 animals in small herds.[30] In our investigation, many persons identified as having Q fever resided at farms that did not meet these criteria; however, it is possible that these people were initially exposed to *C burnetii* when visiting farm 1 to purchase goats.

Culling was not recommended as a means to control *C burnetii* infection in infected herds identified in this investigation. Once infected animals have lived on a property, the resulting environmental contamination will remain as a potential source of transmission even if the herd is depopulated. The bacterium is very hardy and resistant to heat, drying, and many disinfectants; this enables it to survive for long periods (potentially years) in the environment.[1] Results of our investigation indicated that serologic testing is not a reliable method to determine whether specific animals are a potential source of transmission of *C burnetii* to humans or other animals or to determine herd infection status. An animal with a positive serum antibody titer result might not actively shed the organism, and animals might not seroconvert after exposure to *C burnetii* but shed the bacterium by multiple routes. Our results confirmed that testing of biological fluids or feces by PCR assay is a more sensitive indicator of infection and transmission risk.

Herd outbreaks of coxiellosis are challenging to identify and control because animal testing to diagnose *C burnetii* infection in a herd is problematic, and shedding goats, which are often subclinically affected, pose a substantial public health risk. In 2013, the National Association of State Public Health Veterinarians and the National Assembly of State Animal Health Officials released a guidance document[31] that provides recommendations for a coordinated public and animal health response to a Q fever outbreak. Also in 2013, the CDC released guidance for physicians on evaluation and clinical management of Q fever cases.[9] These recent reports are recommended to guide outbreak responders during Q fever investigations. Education is vi-

tal, and goat owners should be informed of the risk for *C burnetii* infection, even in animals that have not been laboratory confirmed as infected, so that measures are taken to control animal transmission and prevent human exposure. Symptoms of human infection should be known so that testing and treatment of exposed humans can be performed immediately, if needed. Persons at high risk for severe or chronic Q fever, such as pregnant women, immunosuppressed persons, or those with valvular heart disease, a vascular graft, or arterial aneurysm,[9] should avoid animal housing areas, particularly birthing areas. Raw milk or raw dairy products should never be used for human consumption or sold.

A well-designed sampling strategy to determine herd infection status in nondairy goat herds requires targeted animal testing and use of PCR assays to test for presence of the organism. If PCR testing is not available and the herd is small (< 30 animals), all animals in the herd should be serologically tested. If the herd size is ≥ 30, serum should be obtained from those animals at highest risk of infection: goats that have recently had an adverse pregnancy event, goats that have recently kidded, pregnant goats, and lactating goats. If PCR testing is available, these same high-risk animals should have samples of feces, vaginal fluids, or milk collected and analyzed. Placental material, even from apparently healthy does with live offspring, can also be used to diagnose infection in a herd through PCR assay or immunohistochemical analysis.

For sample size calculations for serologic testing, a larger sample is required because sensitivity is much lower, compared with that of PCR testing (Table 3). If the herd is predominantly a dairy herd, bulk-tank milk specimen testing via PCR assay is recommended. Whatever strategy is chosen, it is important to note that testing is most useful to evaluate for infection at the herd level, not at the individual-animal level. Since serologic testing was shown to have poor sensitivity and shedding can be intermittent, a negative test result for an individual animal does not confirm that it is not infected.

Quarantines and temporary holds to prevent the sale or movement of potentially affected goats were used in this outbreak as a control measure. The purpose was to allow time for the investigators to perform a trace-forward of all affected farms, educate farmers of the disease risk, perform human and animal testing to determine the scope of the outbreak, and place affected farms under a herd management plan. On farms that pose a high potential risk for human transmission, temporary measures such as quarantine or a temporary hold may be needed to decrease the risk of human transmission during outbreaks. All future purchasers of goats from the infected farm need to be educated of the herd infection status. Control measures, such as a herd management plan, should be implemented prior to lifting a temporary hold or quarantine. During an abortion storm, the sale or transport of goats, particularly pregnant does, from the affected farm should be prohibited.

Control of coxiellosis in livestock in the United States is dependent on a combination of good management practices, appropriate sampling, and easily available diagnostic laboratory testing. Animal vaccination against *C burnetii* has been proven successful as a primary means of Q fever outbreak control in The Netherlands.[30,32] A resolution approved at the 2011 US Animal Health Association meeting recommended that USDA facilitate the importation of animal vaccines against *C burnetii* for investigation and research purposes.[33] Veterinarians need to include coxiellosis in their differential diagnoses if appropriate when investigating neonatal mortality and abortions, particularly in small ruminants.[29] Abortion diagnostic specimens should include placental tissue samples (including cotyledons) if available, as well as maternal samples such as vaginal fluids and milk. State veterinary diagnostic laboratories should provide capabilities necessary for diagnosis of *C burnetii* infection, including PCR assays and immunohistochemical tests, or designate a veterinary reference laboratory to perform this testing. Enhanced diagnostic capabilities will also assist in needed surveillance and research to better understand the geographic distribution of infection and economic impact of this disease in the United States. Increased collaboration between the veterinary and public health communities is required so that potential animal exposures for people with a diagnosis of Q fever are investigated and so that persons in contact with animals confirmed to have coxiellosis, their immediate environment, or their raw products are notified of the transmission risk. *Coxiella burnetii* is a pathogen that resides closely at the human-animal interface. Increased awareness of this challenging zoonosis will lead to increased diagnosis and reporting in both humans and domestic animals.

a. Online Supplement available at http://avmajournals.avma.org/toc/javma/247/12.
b. IDEXX, Liebefeld-Bern, Switzerland.
c. Qiagen QIAamp DNA Mini Kit, Qiagen Inc, Valencia, Calif.
d. Epi-Info, version 3.3.2, CDC, Atlanta, Ga.

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