Epidemiol. Infect. (2016), **144**, 19–24. © Cambridge University Press 2015
doi:10.1017/S0950268815000874

SHORT REPORT
Serological survey using ELISA to determine the prevalence of *Coxiella burnetii* infection (Q fever) in sheep and goats in Great Britain

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Received 30 October 2014; Final revision 13 March 2015; Accepted 7 April 2015; first published online 20 May 2015

SUMMARY

A survey of *Coxiella burnetii* infection (Q fever) in sheep flocks and goat herds in Great Britain was undertaken. A total of 5791 sheep (384 flocks) and 522 goats (145 herds) were examined for *C. burnetii* antibodies using an ELISA. Overall, 53 sheep (37 flocks), and four goats (four herds), tested positive. Estimates of individual animal, between-flock/-herd and within-flock/-herd crude prevalences were 0·9%, 10·2% and 9·0%, respectively, for sheep, and 0·8%, 3% and 26·3%, respectively, for goats. With sheep, the likelihood of an animal testing positive increased with total flock size ($P = 0{\cdot}002$) and number of breeding ewes in the flock ($P = 0{\cdot}021$). It also increased with number of goats within a 10 km radius ($P = 0{\cdot}038$). There was no evidence for spatial clustering of positive herds above that expected by chance alone. No analysis of risk factors was attempted for goats because of the paucity of positives.

Key words: Goats, Q fever, sheep, surveillance, zoonoses.

Q fever is an important zoonotic disease caused by the bacterium *Coxiella burnetii* which has recently been reclassified as belonging to the order Legionellales [1]. Infection is mainly subclinical in animals, although clinical signs (usually abortions, sometimes as outbreaks) are occasionally reported in cattle, sheep and goats [2–4]. Although the economic implications for livestock farmers are usually minimal, Q fever is an important zoonosis and public health concerns have increased markedly following the large human outbreak in The Netherlands. This outbreak resulted in 2357 human cases in 2009 alone, compared to 168 and 1000 in 2007 and 2008, respectively [5]. In people, clinical disease presents with febrile illness, atypical pneumonia and hepatitis although about 60% of cases are asymptomatic. Chronic sequelae can occur, including endocarditis or a chronic fatigue syndrome, and pregnant women are at particular risk of developing complications following Q fever [1].

Human infection has been associated with contact with cattle, sheep and goats, and Q fever is an occupational hazard in high-risk groups, such as livestock farmers or slaughterhouse workers. The main route of zoonotic transmission [1] is by contaminated aerosols from infected animals, especially farmed ruminants; commonly implicated sources include manure, bedding, dust particles and parturition products, including abortions. The outbreak in The Netherlands

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(2007–2010) was attributed to airborne spread from a region densely populated with infected dairy goat herds and led to a major vaccination and culling programme. In 2009, 59% of the notified human Q fever cases lived within a 5 km radius of an infected dairy goat or dairy sheep farm. In comparison, nationally, 12% of the Dutch population live within these zones. In one instance, people living within 2 km of an infected dairy goat farm had a 31 times greater risk of developing Q fever than those living more than 5 km away [5].

In GB Q fever is considered to be endemic. For example, in a survey of cattle herds from England and Wales 21% of 373 dairies showed serological evidence of infection in bulk tank milk samples [6]. However, only a handful of clinical diagnoses of Q fever are currently recorded in farm ruminants each year in GB and human cases often act as sentinels for undetected infection in animals. Thirty-seven human cases were recorded in England and Wales in 2008 [7] and large outbreaks (>20 cases) can also occur in the general population, for example in Cheltenham, England, in 2007 [8]. Other European countries have shown a higher seroprevalence, although different serological tests and cut-off points were used in the individual studies [9]. Within-herd prevalence estimates for European countries with an endemic situation for sheep were 56·9% in Bulgaria, 20·0% in France, and 8·7% in Germany; and for goats it was 40·0% in France [10].

An understanding of the epidemiology of Q fever in animals, especially sheep and goats, is vital to help mitigate the risks to human health, particularly in the light of the recent outbreak in The Netherlands. The small number of clinical incidents of Q fever recorded in animals in GB is unlikely to provide an accurate reflection of prevalence because most infection is subclinical and not detectable without serology (which is rarely undertaken). Consequently recent accurate prevalence data for GB are lacking from the literature. To address this we utilized surplus sera collected from sheep and goats in 2008, for an annual survey of *Brucella melitensis*, to estimate the prevalence and geographical distribution of *C. burnetii* infection in GB and make a preliminary assessment of possible risk factors.

The *B. melitensis* survey comprised randomly selected premises stratified by Animal Health (AH) areas of operational responsibility (AH region). GB is split into 24 regions (16 in England, three in Wales and five in Scotland), and all regions were sampled. Twenty blood samples were randomly collected from breeding ewes/goats per flock/herd, or from all available animals if <20 were present on farm. In total, blood samples were collected from 1353 sheep flocks (23153 animals) and 145 goat herds (522 animals). Additional information collected on the submission forms included the numbers of breeding ewes and goats on the farm and the presence or absence of other livestock species (cattle, pigs, deer, camelids, poultry).

A commercially available ELISA (LSI, Life Technologies, UK) was used to examine a subset of the sera from the *B. melitensis* survey for *C. burnetii* antibodies. This test has recently been comprehensively evaluated within the Animal and Plant Health Agency (APHA) [9] and found to be both fit for purpose and superior to the outdated complement fixation test (CFT) used previously within the APHA. Using APHA TAGS (test accuracy in the absence of a gold standard) data [11], ovine sera gave a sensitivity of 88·8% and specificity of 98·5%; caprine sera had a sensitivity of 91·6% and specificity of 98·9%. The LSI ELISA has now been adopted for routine use within APHA as a replacement for the CFT.

A sample size calculation identified that of the sheep flocks sampled for the *B. melitensis* survey 384 flocks were randomly selected for Q fever testing, in order to estimate the prevalence of *C. burnetii* seropositive flocks in GB with a precision of ±5% and 95% confidence, assuming a true prevalence of about 50% [2, 3]. The sample was stratified according to the number of flocks in each AH region, to ensure a representative geographical spread of samples. Sixteen sera samples were tested from each flock (or all samples from a flock, where fewer than 16 were available) to give 95% confidence of detecting at least one positive animal in an affected flock, based on the sensitivity and specificity previously quoted, and if flock prevalence was as low as 20%.

Since fewer goat samples were collected during the *B. melitensis* survey all available samples were tested for *C. burnetii*. This was sufficient to estimate the individual animal prevalence with a precision of ±1·9% with 95% confidence, if the true prevalence were 5% and clustering by farm were not taken into account. However, with clustering on farms, the precision would be reduced to about ±5·0%

In addition, complete flock/herd sizes were obtained from rapid analysis and detection of animal-related risks (RADAR) [12] for June 2008, and data were also extracted on sheep, goat and cattle density in each of the AH regions, and proximity to a variety

Table 1. *Summary of animal prevalence (AP) and between-holding prevalence (BHP) with confidence intervals (CIs), and estimates of within-holding prevalence (WHP)*

	Samples tested: animals (holdings)	Crude AP (%)	True AP (%)	True AP (95% CI)	Crude BHP (%)	True BHP (%)	True BHP (95% CI)	Estimated WHP (%)
Sheep	5790 (383)	0·92	0	0–0·013	9·66	10·17	8·65–11·7	9·00
Goats	512 (142)	0·78	0	0–0·46	2·82	2·97	1·57–4·37	26·30

of other farm types. Complete flock sizes for 238 (62·0%) of the 384 flocks of sheep and 89 (61·4%) of the 145 herds of goats were obtained from RADAR for June 2008.

Data were used to calculate the true prevalence of *C. burnetii* antibodies in the sheep and goat adult populations using FreeCalc [13], based on the sensitivity and specificity of the ELISA. Subsequently, risk factor analysis was carried out using multilevel logistic modelling in MLwiN 2·1 [14] at the animal level. This allowed the models to account for the hierarchical structure of the data, i.e. individual animals within a holding within the AH region. All potential risk factors that had been recorded on the *Brucella* sample submission form, or that were extracted from RADAR, were initially examined individually in logistic regression models. Sampling dates were sine and cosine transformed to allow for seasonality.

The spatial distribution of the survey results was analysed. R statistical package 2·7·1 (R Development Core Team, Austria) was used for spatial analysis of the sheep, goat and combined species data. Although some holdings were located on islands, for the purpose of analysis the polygon of Great Britain was drawn to include the islands as being attached to the mainland. Spatial analysis was by means of a case-control *K*-function analysis which tests the null hypothesis of an equivalent degree of clustering in positive and negative holdings against an alternative of differential clustering mechanisms in the two groups [15] and was completed using the Splancs library [16]. Spatial heterogeneity of the combined sheep and goat results was also tested via a geo-statistical approach, by plotting variograms using the geoR library [17], to analyse whether spatially close farms have more similar results than expected by chance.

Of the 384 sheep flocks tested, results were returned for 383 (5790 animals); data from one flock (one animal) was unavailable (insufficient sample). In total, 53 animals were positive in 37 flocks. Of the 145 goat herds (522 animals), results were returned for 142 herds (512 animals); three samples were lost after testing for the *B. melitensis* survey. In total, four animals in four herds were seropositive. Animal, between-holding and within-holding prevalence levels were calculated, and the results are summarized in Table 1. Although true animal prevalence for both sheep and goats was not significantly different to 1, all positive samples were re-tested and all remained positive, thus increasing our confidence in the test result. Consequently, all positive results were treated as though truly positive for the remainder of the analysis. It should be noted that the mean number of animals sampled in each of the goat herds was just 3·6, rather than 16. Assuming that where fewer than 16 animals were sampled, the whole flock was sampled, our confidence in disease detection is not affected.

Since within-holding samples were only designed to detect at least one infected animal on a holding, if infection was present, we cannot use the results to directly estimate within-flock prevalence. However, if we assume that crude animal prevalence is accurate, and using true between-holding prevalence, within-flock prevalence can be estimated using the formula: animal prevalence/herd prevalence × 100.

Risk factor analysis was carried out to identify risk factors associated with the likelihood of infection with *C. burnetii*. The likelihood of a sheep testing positive increased with the total number of sheep on a holding, estimated from RADAR [for an increase of 10 sheep on a holding: odds ratio (OR) 1·01, $\chi^2 = 4{\cdot}80$, D.F. = 1, $P = 0{\cdot}028$] and number of breeding ewes (for an increase of 20 breeding ewes: OR 1·01, $\chi^2 = 4{\cdot}81$, D.F. = 1, $P = 0{\cdot}028$) in the flock. However, it should be noted that for the former variable data was only available for 62% of holdings. Mean flock size for sheep testing positive was 1190·0 (95% CI 1117·5–1262·5) compared to 638·6 (95% CI 638·1–639·1) for sheep testing negative. Mean number of breeding ewes on holdings of sheep testing positive was 483·5 (95% CI 458·9–508·1) compared to 257·3 (95% CI 257·2–257·5) on holdings of sheep testing negative.

The likelihood of a sheep testing positive tended to increase with increasing goat density (number of

goats/km^2 in each AH region: OR 2·40, $\chi^2 = 2{\cdot}61$, D.F. = 1, $P = 0{\cdot}106$). Only three sheep holdings also had breeding goats, thus the effect of having breeding goats on the same holding could not be tested. However, the number of goat holdings within a 5 km and a 10 km radius of each sheep farm, as well as the distance to the nearest goat farm, were extracted from RADAR. The likelihood of a sheep testing positive increased with the number of goat holdings within a 10 km radius (OR 1·05, $\chi^2 = 4{\cdot}31$, D.F. = 1, $P = 0{\cdot}038$). Mean number of goat holdings within 10 km on an unaffected farm was 9·2 (95% CI 8·4–10·0) *vs.* 10·2 (95% CI 8·0–12·4) on an affected farm.

Neither presence of cattle on farm, nor cattle or sheep density in AH region, nor distance to the nearest cattle or sheep holding, were significantly associated with the likelihood of being seropositive in any model. Likewise the presence of pigs, or poultry on farm had no significant effect. However, the likelihood of being seropositive did increase with the number of pig holdings within 5 km (OR 1·09, $\chi^2 = 4{\cdot}51$, D.F. = 1, $P = 0{\cdot}034$). Mean number of pig holdings within 5 km on an unaffected farm was 4·9 (95% CI 4·5–5·4) *vs.* 5·9 (95% CI 4·5–7·4) on an affected farm. Too few holdings also had deer or camelids on the premises to examine the effect of these species. While we were unable to obtain numbers of these species surrounding tested holdings from RADAR, the likelihood of being seropositive did increase with the number of holdings with 'other' animals within 10 km (OR 1·08, $\chi^2 = 4{\cdot}37$, D.F. = 1, $P = 0{\cdot}037$). Mean number of other holdings within 10 km of an unaffected farm was 4·7 (95% CI 4·1–5·2) compared to 5·3 (95% CI 3·9–6·7) of an affected farm. There was no evidence of temporal effect from sinusoidal date components of the model.

As only four goats tested positive for Q fever antibodies no risk factor analysis was attempted for this species.

Spatial analysis was carried out on positive holdings. Of the 37 positive sheep holdings, seven were in the Exeter AH region, five in Taunton AH region, five in Galashiels AH region, and five in Carmarthen AH region. The remaining 15 holdings were spread across 11 regions. Two sheep farms had recorded separate County Parish Holding (CPH) references for their holding but identical postcodes. As *K*-function analysis does not allow points at the same location, the x and y coordinates were discretely agitated to move the farms to spatially close but separate locations. The spatial heterogeneity of the sheep data, determined by the *K*-function analysis, was within the simulation estimate envelopes, thus the degree of clustering within positive holdings is no different than that within negative holdings.

All four positive goat holdings were in different AH regions, with one holding each in Taunton AH region, Exeter AH region, Carmarthen AH region and Caernarfon AH region. For the *K*-function analysis of the goat data, a further three data-points were removed from the spatial analysis as they could not be linked to map coordinates. The spatial heterogeneity of the goat data was within the simulation estimate envelopes, thus the degree of clustering within positive holdings is no different than that within negative holdings.

Variogram analysis, using both sheep and goat farm results, assessed whether farms which are spatially close together were more likely to have similar test results (i.e. whether positive holdings tend to be geographically closer together than would be expected by chance). The plot of the analysis results were within the envelopes of the spatially uncorrelated Monte Carlo simulation estimates, and thus the spatial heterogeneity was not beyond that expected by chance.

This comprehensive survey indicated a low seroprevalence of *C. burnetii* infection in sheep and goats in GB in 2008 at both individual animal and between flock or herd levels. To have undertaken a survey of this size specifically for Q fever would have been prohibitively expensive and it must be accepted that there were inevitable limitations because the samples examined for *C. burnetii* were derived from a much larger survey of *B. melitensis* undertaken in 2008, which restricted our ability to collect data specifically appropriate to surveillance for *C. burnetii*. Furthermore, since data were historical by the time testing was complete, follow-up investigations of inconsistencies in data collection were impossible.

The individual animal prevalence was much lower than in The Netherlands where recent data [5] indicated a seroprevalence of 2·4% in sheep ($n = 12363$ samples) and 7·9% in goats ($n = 3409$). These seroprevalence figures reflect the period before and during the early stages of the epidemic, and were lower than recent ELISA prevalence figures reported in other European countries (11·7% in Spain and 8·7% in Germany for sheep; 32·0% in France and 8·7% in Spain for goats) [9]. However, during the outbreak in The Netherlands prevalence rose to 14·8% in sheep and 29·0% in goats [18]. In the present study,

individual animal seroprevalence in goats (0·8%) was lower than sheep (0·9%), which is the opposite of the situation in The Netherlands. The seroprevalence detected in sheep and goats was much lower than that recorded in cattle in England and Wales by Paiba and others [6] or in cattle and sheep in Northern Ireland [4].

Increases in the total flock size and the number of breeding ewes in the flock were significantly associated with an increase in the likelihood of a sheep testing seropositive for *C. burnetii*. We would expect the total number of sheep and number of breeding ewes to be correlated with each other, and the fact that they were both similarly associated with the likelihood of being seropositive, increases our confidence in this result. Logically, large flocks might be expected to be more likely to have at least one infected animal by chance alone. However, large flocks are also arguably more likely to be infected than small flocks because they purchase a larger number of breeding replacements, possibly from a wider range of sources, thereby increasing the risk of introducing infection. Furthermore, testing of replacements is not routinely undertaken for *C. burnetii* because the infection is not considered to be of economic significance to producers.

Our findings suggested that sheep from areas with a high density of goats were more likely to be positive for *C. burnetii* antibodies. Further analysis showed that flocks with more goat herds within a 10 km radius were significantly more likely to test positive, which may suggest that goat herds are a reservoir of this infection. In The Netherlands there is a much higher abortion rate in infected goat herds than infected sheep flocks. However, further studies are needed to support any hypothesis about the relationship between infection in sheep and goats.

Despite the fact that 14 (34%) of the 41 positive sheep or goat holdings were located in two adjacent AH regions, there was no statistical evidence for between-herd clustering. These two regions were in areas of high flock density so the findings were much as expected. The goat analysis had few positives which may have reduced the capacity of the spatial analysis to identify any patterns. We have no information about the current prevalence of infection in cattle herds, so it is impossible to know whether this might have had an effect although we found no association with cattle density.

Most goat herds in the study had only small numbers of animals (average of 3·6 samples taken per herd), which reduces our ability to be confident of disease freedom within each herd. However, the mean goat herd size in June 2008, according to the UK agricultural survey, was just 10·6 animals per herd. Thus, although we are aware that there are also some very large milking goat herds (>500 does) in GB, this sample may be representative. It would be interesting to determine whether the prevalence of infection in very large herds is greater than in the general goat herd population.

This survey only detected the presence of *C. burnetii* antibodies (i.e. evidence of past exposure to infection) and not the initial stages of active infection. However, seropositive animals are likely to have been sources of infection to other animals and people. In its spore-like form [19], the organism is also very resistant in the environment. Hence the 2008 data are likely to be fairly representative of the present situation in GB regarding the presence of *C. burnetti*. Although we examined the data for any effect of sampling date and consequent evidence of seasonality, the absence of significant effect does not mean that this infection is not seasonal since infections that were active during any high-risk seasons would still have been detected in later samples and prevalence would have been cumulative throughout the year.

Reliable current information on the prevalence of *C. burnetii* infection in GB has been significantly lacking from the GB literature for some time and the findings presented here are reassuring in view of the major human and veterinary public health challenges from Q fever facing The Netherlands. Our findings, together with the continued low annual number of cases, and a different structure to the livestock industry (particularly the absence of dense concentrations of large goat herds in areas of high human population), suggest a low likelihood of GB experiencing a Q fever outbreak on the same scale as The Netherlands. However, the unexpectedly low seroprevalence demonstrates a naive sheep and goat population that may be prone to seeding rapid clonal expansion in a similar way to that seen in the naive goat population prior to The Netherlands outbreak.

ACKNOWLEDGEMENTS

The work was funded by Defra under project OZ0149. The authors thank colleagues Martin Lenkersdorf for help with data extraction from RADAR and Beth Morris-White for entering the data for analysis.

DECLARATION OF INTEREST

None.

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