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**SID 5**

**Research Project Final Report**

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### Project identification

1. **Defra Project code**
   - OZ0149

2. **Project title**
   - Survey to determine the sero-prevalence of infection with Coxiella burnetii (Q fever) in sheep flocks and goat herds in Great Britain.

3. **Contractor organisation(s)**
   - Veterinary Laboratories Agency,
     Woodham Lane,
     New Haw,
     Weybridge,
     Surrey,
     KT15 3NB.

4. **Total Defra project costs**
   - £ 47,996 (agreed fixed price)

5. **Project:**
   - **start date** ............ 01/12/2009
   - **end date** ............. 30/09/2010
6. It is Defra’s intention to publish this form. 
Please confirm your agreement to do so. ............................................YES X NO X

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a “No” answer.

In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Q fever is a disease of animals and people caused by infection with the bacterium *Coxiella burnetii*, occasionally causing abortions in sheep, goats and cattle, usually without significant economic impact. However, it is a public health issue since outbreaks can occur in people following aerosol exposure to contaminated material from infected farms, and there has been increasing concern following the large recent human outbreak in the Netherlands associated with large infected milking goat herds.

A serological survey was undertaken to provide accurate information on the proportion of infected sheep and goats in GB. This information is not currently available in the literature and the survey was essential in order to assess present disease risks.

The survey made use of retained surplus sera available from a major structured random survey of 1,353 sheep flocks (23,153 animals) and 145 goat herds (522 animals) carried out by Animal Health in GB in 2008 to assess the prevalence of an unrelated infection (*Brucella melitensis*).

The objectives were: to estimate the proportion of sheep flocks and goat herds in GB with serological evidence of exposure to *C. burnetii* (Q fever); to investigate risk factors for *C. burnetii* and to determine whether there was any evidence of spatial over-dispersion of seropositive flocks/herds.

The survey used an ELISA serological test recommended by the European Food Safety Authority (EFSA).

383 randomly selected flocks of sheep (5790 animals), stratified by Animal Health Office region, and 142 goat herds (512 animals) were tested for *C. burnetii* infection by ELISA.

Fifty-three sheep and 4 goats tested positive in 37 flocks and 4 herds respectively. The crude individual animal prevalence for sheep and goats was 0.915% and 0.781% respectively. True prevalence was not significantly different from zero, with 95% confidence intervals 0 – 0.131% and 0 – 0.463% for sheep and goats respectively. All positive samples were retested and remained positive after retesting which increased confidence in the test results, and the crude prevalence.

Crude sheep flock prevalence was 9.66% (37/383) and 2.83% (4/142) for goat herds. For sheep the true flock prevalence was 10.2% (95% CI 8.65 – 11.7%), and it was 2.97% (95% CI 1.57 – 4.37%) for goat herds.

The risk factors affecting the likelihood of testing positive for *C. burnetii* were investigated for sheep but
there were insufficient positives in the goats to perform a similar analysis. The likelihood of a sheep testing positive increased with total number of sheep (for an increase by 10 sheep on holding OR = 1.01, $\chi^2 = 4.80$, df = 1, $p = 0.028$), and number of breeding ewes (for an increase of 20 breeding ewes OR = 1.01, $\chi^2 = 4.81$, df = 1, $p = 0.028$) in the flock. This could be because larger flocks may have a greater turnover of incoming breeding replacements than small flocks and an increased probability of introducing *C. burnetii* with carrier animals.

The likelihood of sheep testing positive tended to increase with goat density (number of goats per square km in each AH region; OR = 2.40, $\chi^2 = 2.61$, df = 1, $p = 0.106$). The likelihood of a sheep being tested positive increased with number of goat holdings within a 10km radius (OR = 1.05, $\chi^2 = 4.31$, df = 1, $p = 0.038$) suggesting that goat herds could be a reservoir of infection.

The spatial distribution of the results was analysed and no evidence was found to suggest clustering of positive holdings.

The small sample size of the goat survey may have affected accurate estimation of disease prevalence and it prevented examination of risk factors and spatial clustering. Submissions for many of the sheep flocks did not include the required number of samples; although we attempted to verify this and exclude flocks which appeared to have been under-sampled, we cannot be sure how this may have affected the representativeness of the survey.

The survey detected past exposure to *C. burnetii* (as of 2008) but by its very nature, the organism tends to persist in infected animals and their environment, so it is likely that these results are a fairly accurate reflection of the situation in 2010.

In conclusion, this survey indicated a low prevalence of serological evidence of exposure to *C. burnetii* in sheep and goats in GB and, together with a different structure to the industry in GB compared with the Netherlands, suggest that a large ongoing Q fever human outbreak from contact with infected goat herds is unlikely. The findings are being prepared for publication in a peer reviewed journal.

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### Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

### Background

This serological survey was undertaken because there is no current accurate published information on the prevalence of *Coxiella burnetii* infection (Q fever) in farm livestock in Great Britain. This bacterial infection does not usually cause clinical disease in animals although it may occasionally cause abortions and a few outbreaks are recorded in farm animals in GB each year. However, it is an important public health concern because it is a zoonotic disease and farm ruminants (sheep, goats and cattle) are the usual source of human infection. Q fever can cause a flu-like illness in people but there may also be more serious consequences, especially in pregnant women and people with existing heart disease. Although it is mainly an occupational hazard in high risk groups such as livestock farmers, veterinary surgeons and abattoir workers, large human outbreaks can occur through aerosol exposure from infected farm livestock, including contaminated bedding and discharges from abortions and normal parturitions. There have been a few large outbreaks in people in GB in recent years but the large current outbreak in the Netherlands linked to dairy goats has provoked increased concern.
Approach and methods

Instead of undertaking the survey by visiting and blood sampling animals on randomly selected farms (which would have been prohibitively expensive), the survey made use of retained surplus sera available from a major random survey of 1,353 sheep flocks (23,153 animals) and 145 goat herds (522 animals) carried out by Animal Health (formerly State Veterinary Service) in GB in 2008 to assess the prevalence of an unrelated infection (*Brucella melitensis*).

The stated objectives were: to estimate the proportion of sheep flocks and goat herds in GB that showed serological evidence of exposure to *C. burnetii* (Q fever); to test whether any of the exposures recorded on the questionnaire for the *B. melitensis* survey were associated with increased risk of a flock or herd being seropositive for *C. burnetii*; to determine whether there was any evidence of spatial over-dispersion of seropositive flocks/herds.

The survey used an Enzyme-linked immunosorbent assay (ELISA) serological test which had been comprehensively evaluated within the Veterinary Laboratories Agency and found to be superior to tests used previously. Sheep and goat sera gave sensitivities of 88.8% and 91.6% and specificities of 98.5% and 98.9% respectively. ELISA is the test of choice as recommended by the European Food Safety Authority (EFSA).

After filtering the *B. melitensis* survey samples, 383 flocks of sheep (5790 animals) were randomly selected, stratified by Animal Health Office region, and tested for *C. burnetii* infection by ELISA. Because of their reduced numbers, all of the goat herds were tested for *C. burnetii*. Samples were no longer available for three herds, thus 142 herds (512 animals) were eventually tested. Data were entered into a Microsoft Access database, and prevalence of *C. burnetii* antibodies was investigated.

Main findings

Fifty-three sheep and four goats tested positive in 37 flocks and 4 herds respectively. The crude individual animal prevalence for sheep and goats was 0.915% and 0.781% respectively.

FreeCalc was used to calculate the true prevalence, taking into account test sensitivities and specificities detailed above. Neither prevalence was significantly different from zero, with 95% confidence intervals 0 – 0.131% and 0 – 0.463% for sheep and goats respectively. All positive samples were re-tested, and all remained positive, thus increasing our confidence in the test results, and the crude prevalence.

For sheep, crude flock prevalence was 9.66% (37/383), while for goats, crude herd prevalence was 2.83% (4/142). If we are at least 95% confident of detecting at least one positive animal in an infected flock, and assuming a ‘positive flock/herd’ is always a true positive, then for sheep the true flock prevalence is 10.2% (95% CI 8.65 – 11.7%), and for goats the true herd prevalence is 2.97% (95% CI 1.57 – 4.37%).

The risk factors affecting the likelihood of testing positive for *C. burnetii* were investigated for sheep. There were too few positive samples among the goats to perform a similar analysis. MLwiN 2.2 was used to create multilevel logistic models representative of the hierarchical structure of the data (animals within flocks). The likelihood of a sheep testing positive increased with total number of sheep (for an increase by 10 sheep on holding OR = 1.01, \( \chi^2 = 4.80, df = 1, p = 0.028 \)), and number of breeding ewes (for an increase of 20 breeding ewes OR = 1.01, \( \chi^2 = 4.81, df = 1, p = 0.028 \)) in the flock. Large flocks might be expected to have a greater probability of having at least one infected animal. However, sampling was not proportional to flock size, thus if infection and sampling were random, we would not have been more likely to sample an infected animal. This suggests that there may be a real effect of flock size on likelihood of exposure to *C. burnetii*, perhaps because larger flocks may have a greater turnover of incoming breeding replacements than small flocks, thus having an increased probability of *C. burnetii* introduction with carrier animals.

The likelihood of a sheep testing positive for Q fever antibodies tended to increase with goat density (number of goats per square km in each AH region; OR = 2.40, \( \chi^2 = 2.61, df = 1, p = 0.106 \)). To investigate this further the number of goat holdings within 5km and 10km of each sheep holding was extracted from the Defra maintained data warehouse RADAR (Rapid Analysis and Detection of Animal-related Risks). The likelihood of a sheep testing positive increased with number of goat holdings within a 10km radius (OR = 1.05, \( \chi^2 = 4.31, df = 1, p = 0.038 \)). This may be indicative of the importance of goat herds as a reservoir for Q fever.

The spatial distribution of the results of the survey was analysed using a case-control K-function analysis to test 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. Spatial heterogeneity of results was also tested via a geo-statistical approach, to analyse whether spatially close farms have more similar results than expected by chance. No evidence was found to suggest clustering of positive holdings.

Reliability of findings
The ELISA used here (LSI) was fully validated within VLA before the survey and was well suited to testing large numbers of serum samples.

The small sample size for the goat survey may have affected our ability to accurately estimate disease prevalence, and certainly to examine risk factors and spatial clustering. Our sample comprised many small herds, with a mean of 3.6 samples collected from each herd – slightly smaller than the average GB herd size which, in 2008, was 10.6 animals.

Submissions for many of the flocks (as derived from the B. melitensis survey) did not include the required number of samples. In the event that a flock did not contain 20 animals, all sheep should have been sampled. Although we attempted, through comparison with RADAR, to verify this and exclude flocks which appeared to have been under-sampled, we cannot be sure how this may have affected the representativeness of the survey.

It should be noted that this survey only detected past exposure to C. burnetii (as of 2008) and not current active infection. However, by its very nature, C. burnetii tends to persist in infected animals and their environment, so it is likely that these results are a fairly accurate reflection of the situation in 2010.

Meeting the objectives

Overall, the survey met the objectives and was successfully achieved on a modest budget by making the best use of existing resources. The main confounding issues are outlined above.

Main implications of the findings

The results of this serological survey using a fully validated Q fever ELISA indicated a low prevalence of infection in sheep and goats in GB. This was less than anticipated based on the findings from Northern Ireland and a historical serological survey using bulk milk samples carried out in dairy herds in England and Wales. Significantly, the animal seroprevalence in sheep and goats was somewhat less than that recorded in the Netherlands using 2008 sera (2.4% and 7.9% respectively). These factors, together with a different structure to the goat industry in GB compared with the Netherlands, suggest that a large ongoing human outbreak from contact with infected goat herds is unlikely to occur in GB.

Actions arising

Limited preliminary results from this survey have been discussed in several fora including the multi-disciplinary Human Animal Infections and Risk Surveillance (HAIRS) group and a recent meeting of the Goat Veterinary Society. The low prevalence of infection has since been supported by the results of a VLA survey carried out under a Defra funded surveillance project (FZ2100) using a sensitive PCR to detect Coxiella burnetii in randomly selected abortion material submitted to VLA Regional Laboratories.

Suggestions for further work

This survey covered sheep and goats in GB but similar information is not currently available for cattle herds. Validation of the ELISA for use with bulk milk samples would provide a potentially cost-effective method of surveying dairy cattle herds and also monitoring goat herds (especially large milking herds) and sheep flocks to detect any increased risk to human health. It would also be useful to investigate further the possible role of goat herds acting as a reservoir of infection for sheep flocks as tentatively hypothesised here. This study also highlights the possibility of carrying out a more extensive study of the risk factors which predispose a farm to infection with C. burnetii.

Publication of the survey results

The findings from this survey are currently being prepared for publication in a peer reviewed journal.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Paper intended for publication in Epidemiology and Infection:
Serological survey using ELISA to determine the prevalence of Coxiella burnetii infection (Q fever) in sheep and goats in Great Britain