Investigating the prevalence of *Coxiella burnetii* infection in a dairy herd in Scotland

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Introduction

Coxiella (*C.*) *burnetii* is an obligate intracellular, gram-negative bacterium and the etiologic agent of Q fever in humans and coxiellosis in animals. The relationship between coxiellosis and reproductive disease in cattle, such as uterine infections, is slowly becoming better understood (Muskens, Van Maanen and Mars, 2011). Previous studies have linked *C. burnetii* infection to reproductive diseases in dairy cattle breeds (Ullah et al. 2022). *Coxiella burnetii* has also been identified as a presumptive etiology of abortion in cattle by qPCR testing using placental tissues, vaginal swabs, and exterior foetal swabs (Thomas et al., 2022). The prevalence of this pathogen in UK dairy herds has been found to be 79.7% and 28.6% based on bulk tank milk ELISA and PCR tests, respectively (Velasova et al., 2017).

Although *C. burnetii* is shed mainly via birth products (e.g., birth fluids and placenta) in ruminants (Figure 1), it can also be shed via vaginal mucous, milk, faeces and semen (Guatteo et al., 2011). Hence, using alternative samples aside from birth products can be viable for diagnosing coxiellosis when placental or foetal tissues are not available.

Figure 1. Post partum cow attending to newborn calf.

Study Aim

This study aim was to determine the prevalence of *C. burnetii* on a commercial dairy farm in Scotland with previous history of *C. burnetii*. Longitudinal sampling will serve to quantify the number of individuals shedding *C. burnetii* DNA.

Materials and Methods

A 900-cow commercial dairy farm in Scottland, UK was recruited in this study following initial positive screening for *C. burnetii* via bulk tank milk PCR (under University of Glasgow ethics number EA34/22).

Vaginal swabs were collected from cows within seven days after parturition. Before sampling, the vulva of the cow was thoroughly cleaned using a solution of Hibiscrub® (Regent Medical Ltd., UK) and subsequent drying with a paper towel (Star Tissue Ltd., UK), a sterile collection swab was then inserted through the vaginal to collect the vaginal fluids and immediately stored in an sterile Eppendorf tube with 1x DNA/RNA Shield® (Zymo Research).

The vaginal swabs were analyzed with qPCR using an IS1111 assay (Quantabio, US) on extracted bacterial DNA. Samples were tested using 40-45 cycles following standard Qiagen RotoGene operating procedures.

Data was entered into Microsoft Excel (Microsoft Corporation) and exported in R Studio (RStudio Team, 2020) for further statistical analysis.

Results

A total of 346 vaginal swabs were collected between December 2022 to August 2023 and tested in seven separate 'Runs'. Vaginal swab qPCR results indicated that 95.5% (CI 95%; 92.7-97.4%) of the cows were positive for *C. burnetii* (Cycle threshold (Ct) ≤ 40) and 90.6% (CI 95%; 87.1-93.5%) of Ct scores indicated that these cows were actively shedding *C. burnetii* (Ct ≤ 35). A total of 64 (18.2%) (CI 95%; 14.3-22.6%) samples had Ct scores (Ct ≤ 27) attributable to clinical disease (Figure 2). A greater (P<0.05) proportion of primiparous cows were positive (Ct ≤ 35) for *C. burnetii* than multiparous cows (Figure 3).

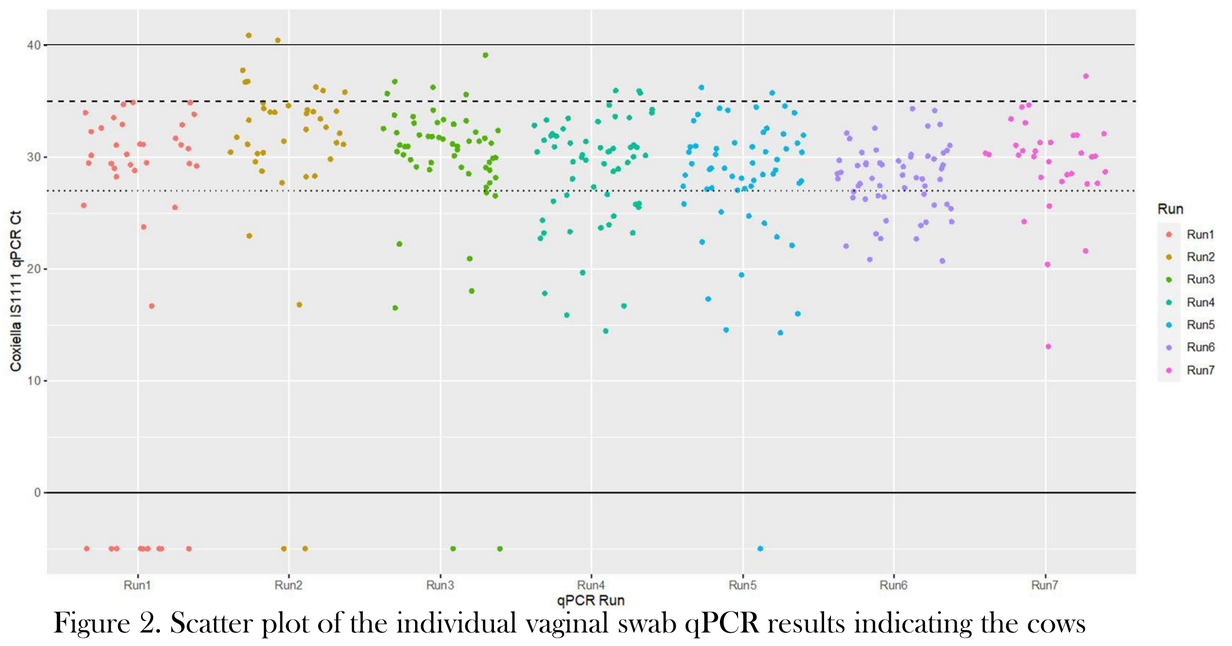

Figure 2. Scatter plot of the individual vaginal swab qPCR results indicating the cows positive for *C. burnetii* (Ct ≤ 40, solid line), cows that were actively shedding *C. burnetii* (Ct ≤ 35; dashed line), and cows that *C. burnetii* could be attributable to clinical disease (Ct ≤ 27; dotted line)

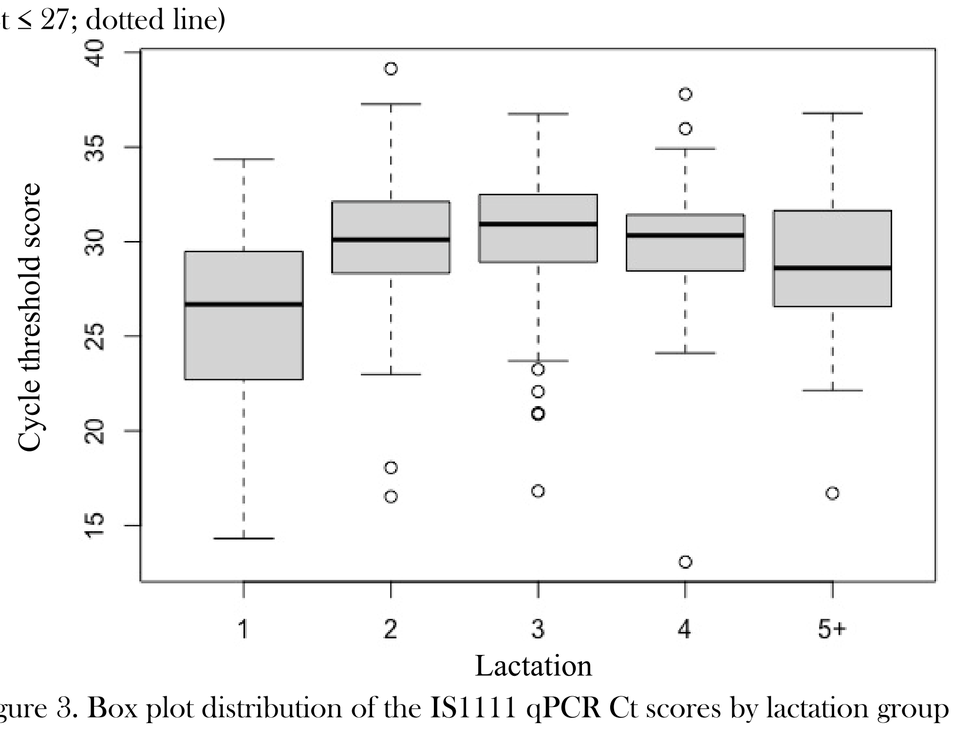

Figure 3. Box plot distribution of the IS1111 qPCR Ct scores by lactation group (Median values, solid lines in boxes)

Discussion and Conclusion

The initial IS1111 qPCR results indicate that *C. burnetii* is endemic to the farm habitat, with various Ct scores shown from individuals tested. The effect of parity in the qPCR results may be due to naïve heifers having an initial exposure to *C. burnetii* after their introduction in the adult herd. The qPCR test found individuals with Ct's ≤ 27 which can be attributable to clinical disease. However, further analysis is required to determine impacts on production as well as correlate infection status to reproductive dysfunction.

References

1. Agerholm, J., 2013. *Coxiella burnetii* associated reproductive disorders in domestic animals - a critical review. *Acta Vet Scand* 55:13
2. Guatteo, R., Seegers, H., Taurel, A.F., Joly, A. and Beaudeau, F., 2011. Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. *Veterinary microbiology*, *149*(1-2), pp.1-16.
3. Thomas, K., Kibona, T., Claxton, J.R., de Glanville, W.A., Lankester, F., Amani, N., Buza, J.J., Carter, Ryan., Chapman, G.E., Crump, J.A., Dagleish, M.P., Halliday, J.E.B., Hamilton, C.M., Innes, E.A., Katzer, F., Livingstone, M. Longbottom, D., Millins, C., Mmmbaga, B.T., Mosha, V., Nyarobi, J., Nyasebwa, O.M, Russell, G.C, Sanka, P.N., Semango, G., Wheelhouse, N., Willett, B.J., Cleveland, S., Allan, K.J., 2022. Prospective chort study reveals unexpected aetiologies of livestock abortion in northern Tanzania. *Scientific Reports, 12.*
4. Ullah, Q., Jamil, T., Muhammad, S., Iqbal, M., Neubauer, I., 2022. Q fever – A Neglected Zoonosis. *Microorganisms.* 2022; 10(8):1530. https://doi.org/10.3390/microorganisms10081530
5. Muskens, J., Van Maanen, C. & Mars, M. H., 2011. Dairy cows with metritis: Coxiella burnetii test results in uterine, blood and bulk milk samples. Veterinary Microbiology 147(1-2), pp. 186-189
6. Velasova *et al.* (2017) Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain. *J Dairy Sci* 100(11) pp9215-9233; DEFRA (2021)