Coxiella burnetii shedding during the postpartum period and the impact on reproductive disease burden: A cross-sectional investigation in a single Scottish dairy herd

Griffin McCleod Weller

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School of Biodiversity, One Health and Veterinary Medicine

College of Medical, Veterinary & Life Sciences, University of Glasgow,

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ABSTRACT

Coxiella burnetii is a poorly understood pathogen with a wide geographical distribution across a plethora of host species. Impact on cattle reproductive health and herd fertility has been indicated internationally, but detailed investigations of the prevalence and impact on production in the UK are scarce. The gap in knowledge of the clinical impact *C. burnetii* infections has on dairy cattle is quite large with varied clinical impacts across herds, so a study elucidating the behaviour and effects of *C. burnetii* in British herds is required. The objectives of this study were to determine the within-herd prevalence of *C. burnetii* on a commercial dairy farm and assess the relationships between pathogen shedding and key performance indicators (KPI) and reproductive health. A 900-cow dairy farm in Scotland was recruited in this study following positive screening for *C. burnetii* by PCR and ELISA. Vaginal swabs were collected from postpartum cows during checks performed within seven days of parturition. DNA extracts were tested by qPCR for *C. burnetii* using an *IS1111* assay. The estimated log(*n* genomes per reaction) was calculated for all samples. Mixed effect Gaussian generalized linear models with identity link function were used to identify variables associated with higher (or lower) concentrations of bacterial DNA. The relationships between cow production and clinical data and this outcome were assessed in uni- and multivariable models. A total of 324 swabs were collected between December 2022 and July 2023, of which 310 (95.7%) had detectable *C. burnetii* with Ct ≤ 40. Cows shedding higher loads of bacterial DNA were more likely to be primiparous and to have experienced one or more negative gestational/obstetric outcomes including abortion, stillbirth, premature delivery, prolonged gestation and/or dystocia. Bacterial load did not correlate significantly with the days postpartum on which individuals were sampled. These findings indicate *C. burnetii* infections have negative impact on the health status and fertility of herd members in the sample population. Further investigation should take place to assess ongoing herd-wide effects of *C. burnetii* infections to better inform possible treatment and prevention strategies.

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INTRODUCTION

Historical Context of *Coxiella burnetii*

Coxiella burnetii is an obligate intracellular, gram-negative bacterium, a category B biological weapon and the causative agent of *C. burnetii* infections in animals and Q fever in humans (OIE, 2018, WHO, 2021). In the 1930s, *C. burnetii* was associated with an outbreak of febrile illness in laboratory workers unpacking samples sent from Queensland, Australia, like the flu 'Query' fever- first seen in abattoir workers in Australia (Derrick, 1937, Dyer, 1939). *R*ickettsia *rickettsia* was the name given to this new agent isolated from local ticks simultaneously in Nine Mile, Montana, USA. The name was subsequently changed to *Rickettsia burnetii* after isolation from inoculated mice spleen at the Laboratory of Microbiology and Pathology, Queensland Health Department in Brisbane, Australia (Dyer, 1939). In Nine Mile, Montana, USA, researchers were also trying to find the causative agent of a disease called Rocky Mountain Spotted Fever spread by ticks, with symptoms like those of 'Q-fever '(Bengtson, 1937). Relation of these events occurred by coincidence when a researcher in Montana developed symptoms after handling Q Fever samples from a Brisbane lab (Burnet, 1941). During World War II, thousands in Europe from 1939–1945 were subject to exposure to the agent resulting in outbreaks of 'Balkangrippe'. Soldiers would often become ill after sleeping on contaminated straw and bedding from housing for small and large ruminants (Anderson et al., 2005, Spicer, 1978).

Since its discovery, *C. burnetii* has evaded diagnosis as the etiologic agent of many cases of Q fever and/or *C. burnetii* infections due to the polymorphic and polyphasic nature of this organism (Böttcher et al., 2011, Abnave et al., 2017, Hotta et al., 2002, Schramek et al., 1985). This may explain the original and mistaken classification as *Rickettsia* until 1948. Researchers dubbed this pathogen with the genus and subgenus *C. burnetii* 13 years after the initial discovery (Philip, 1948).

Morphology and virulence of *Coxiella burnetii*

The bacteria *C. burnetii* is pleiomorphic, so it is able alter its shape and size in response to environmental conditions (Burnet, 1941, Derrick, 1937, Maurin and Raoult, 1999). The hardiest and most virulent morphology of the bacteria, known as the small cell variant (SCV) is shed by infected hosts. It is metabolically inactive and has a spore-like cell wall (Abnave et al., 2017, Schramek et al., 1985). *Coxiella burnetii*, once shed, exists in the environment without the ability to form a true spore. Regardless, it can withstand exposure to harsh environmental conditions and various chemical cleaning agents (Kersh et al., 2010, Nusinovici et al., 2017). This allows the SCV to survive in sheds, pastures or dust piles for months to years (Kersh et al., 2010). The metabolically active homologue, the large cell variant (LCV), evades the immune system by living and replicating within the acidic phagolysosomes of somatic cells, most often macrophages and trophoblasts (Kersh et al., 2010, Nusinovici et al., 2017). Individual bacteria may be found in any phase whether it is a LCV or a SCV (Schramek et al., 1985, Hotta et al., 2002).

Bacterial antigens, like those found on the surface of *C. burnetii,* contribute to the virulence of pathogens. Like *Chlamydia spp., C. burnetii* can alter its surface antigens, making it a polyphasic bacterium (having ≥ two phases). The different phases are described based on morphological alterations to outer membrane components. Phase I is known as the rough form and Phase II the smooth-rough form (Schramek et al., 1985). Phase I *C. burnetii* can be distinguished as having a larger and complete LPS structure with an O-antigen. Phase II is known as the smooth form due to LPS truncation that occurs after deletion of the O-antigen (Schramek et al., 1985). The truncated LPS found in Phase II is the avirulent phase (Abnave et al., 2017, Hotta et al., 2002). Findings associated with detection *C. burnetii* Phase II antibodies via phase-specific ELISA tests in heifers (1-2 years old) included detection of *C. burnetii* DNA using PCR on placental tissue (Böttcher et al., 2011). Unlike in human Q fever cases, chronic and acute forms of the disease have not been as clearly defined for ruminants with *C. burnetii* infections (WHO, 2021, OIE, 2018). SCV with Phase I LPS cause acute pro-inflammatory host responses like phagocytosis of pathogens and upregulation of cytokine production. *Coxiella burnetii* with phase I LPS become immunosuppressive when the pathogen resumes metabolic activity. Active metabolic bacteria are identifiable using the presence of LCVs within a Coxiella-containing vacuole (CCV) (Weber et al., 2013).

Geographic and host distribution

Coxiella burnetii is endemic in every country apart from New Zealand and is able to infect several species including most mammal species (Kersh, 2023, WHO, 2021, OIE, 2018). It is a pathogen of concern in countries like Australia, the Netherlands, the United Kingdom, France, Spain, Iran, Tanzania, Brazil, Germany and Poland (Burnet, 1936, Connolly, 1968, Roest et al., 2013, Vanderburg et al., 2014, Piñero et al., 2014, Garcia-Ispierto et al., 2015, DAERA, 2016, OIE, 2018, DEFRA, 2021). *Coxiella burnetii* can be isolated from arthropods, reptiles, amphibians, birds, fish and mammals, though ruminants act as the primary reservoir in cases of zoonosis (Dyer, 1939, Plommet, 1973, Guatteo et al., 2011a, Connolly, 1968, Rabaza et al., 2021, Schack et al., 2014, Thomas et al., 2022, Mohabati Mobarez et al., 2022, Szymańska-Czerwińska et al., 2019).

In Spain, there is growing suspicion of wildlife species contributing to the cycle of *C. burnetii* infections within ruminant herds that can contribute to Q fever infections in human populations during zoonotic outbreaks (González-Barrio et al., 2021, Wilson et al., 2010, Beaudeau et al., 2021). Infections from *C. burnetii* are disseminated impacting various hosts, namely due to its ability evade innate and adaptive immune defenses across species (Sireci et al., 2021, Schramek et al., 1985, Böttcher et al., 2011, Van Schaik et al., 2013)

Transmission

Infections in mammals are mostly contracted through inhalation of aerosols or particulates contaminated with *C. burnetii*, though infections from ingestion of contaminated materials, like milk, and haematogenous infections following tick bites represent a smaller portion of *C. burnetii* infections (Nusinovici et al., 2017, Schimmer et al., 2014, Wilson et al., 2010). In ruminants, the postparturirient period poses the highest risk for transmission other herd members, due to the high concentration of *C.burnetii* shed within birth products (Abnave et al., 2017, Schramek et al., 1985, Connolly, 1968, Spicer, 1978, Guatteo et al., 2006, Roest, 2011, Schimmer et al., 2014). Depending on environmental conditions, dried and contaminated bodily fluids, like afterbirth, can aerosolize into dust particulates where they can spread over long distances (Clark and Soares Magalhães, 2018).

In epidemics of community acquired pneumonia (CAP) caused by *C. burnetii*, farms as far as 18 km from non-farming communities were cited as the source of these zoonotic infections (Roest, 2011). Due to its small size, *C. burnetii* is able to aerosolize from contaminated material, such as afterbirth from livestock, and spread far beyond the initial contamination site (Maurin and Raoult, 1999, Wilson et al., 2010, Schack et al., 2014, Beaudeau et al., 2021). Abortion storms often precede acquired community infections like CAP; most disseminate from small ruminant farms (Connolly, 1968, Roest, 2011, Clark and Soares Magalhães, 2018). Goats from small dairy farms were the source of nearly 4,000 CAP cases in the Netherlands during the period of 2007-2011, whereas the yearly average up to 2006 was sixteen diagnoses per anum (Roest, 2011, Wilson et al., 2010, Maurin and Raoult, 1999). Few CAP-causing isolates from the Q fever epidemic in the Netherlands were traceable to dairy cattle (Schimmer et al., 2014). Risk factors for acquiring Q fever amongst dairy cattle farm staff in the Netherlands included: Recent contact with afterbirth, contact with pigs and being male. Several protective factors were also identified including, strict adherence to glove-use during and around calving as well as the use of automated milking systems (Schimmer et al., 2014).

Workers in the agricultural sector are oftenhave the highest risk of acquiring Q fever, for zoonotic cases account for nearly all diagnosed cases of Q fever and human to human transmission accounts for a small portion of reported cases (Roest, 2011, Nusinovici et al., 2017, Clark and Soares Magalhães, 2018, Pouquet et al., 2020, Beaudeau et al., 2021, Kersh, 2023). Isolated cases of sexually transmitted *C. burnetii* infections have been documented both in humans and rodents, as well as isolation of *C. burnetii* from bull semen (Milazzo et al., 2001, Kruszewska, 1997, Kruszewska and Tylewska-Wierzbanowska, 1993).

Precipitation frequency, precipitation volume and topography of the area were risk factors found to contribute to the likelihood of spreading the bacteria to human settlements or other farms in many countries including Germany, the Netherlands and the UK (Roest, 2011, Nusinovici et al., 2017, Clark and Soares Magalhães, 2018, Pouquet et al., 2020, Beaudeau et al., 2021). Additional risk factors including wind speed and nearby production systems influence the risk of community members acquiring Q fever, though human infections from cattle are

considered to be rare occurances (Nusinovici et al., 2017, Clark and Soares Magalhães, 2018, Beaudeau et al., 2021).

Impacts and global burden

For decades, the burden laid upon the agricultural sector by *C. burnetii* has been underestimated, as sourcing tests with high enough sensitivity and specificity to diagnose *C. burnetii* infection within herds remains difficult (OIE, 2018). Much of the literature on *C. burnetii* infections in cattle indicates either unclear or severe impact on reproductive health (see Coxiella burnetii infections in Cattle). The vast majority of attention on the *C. burnetii* is dedicated to the zoonotic potential, especially for populations involved with the agricultural sector or in nearby settlements(Connolly, 1968, Spicer, 1978, Penttila, 1998, Maurin and Raoult, 1999, Guatteo et al., 2006, Wilson et al., 2010, Vanderburg et al., 2014).

The polyphasic and pleomorphic nature of this pathogen contributes to the diagnostic challenges which make investigation of *C. burnetii* infections even more difficult (Burnet, 1936, Bengtson, 1937, Burnet, 1941, Hotta et al., 2002, Duron, 2015, OIE, 2018). Histopathology, the primary diagnostic used to diagnose *C. burnetii* infection, lacks the sensitivity and specificity of molecular diagnostic tests (Hotta et al., 2002, Derrick, 1937, Dyer, 1939, Philip, 1948, Schramek et al., 1985, Maurin and Raoult, 1999, Roest, 2011, OIE, 2018). Cases of *C. burnetii* infections and Q fever are often acute or subclinical, with 60% of diagnosed Q fever in humans cases being asymptomatic (Kersh, 2023). The non-specific nature of clinical signs associated with *C. burnetii* infections makes differentiation from other pathogens difficult (see sections Coxiella burnetii infections in Cattle and Q fever) (Derrick, 1937, Plommet, 1973, Maurin and Raoult, 1999, Dyer, 1939).

The impacts *C. burnetii* infection have on key performance indicators (KPI) in livestock appear to vary by species, production system and country. While a substantial body of evidence has documented the reproductive impacts of infection in small ruminants, the evidence for reproductive impacts in dairy cattle is less robust (Martinov, 2008, Barlow et al., 2008, Guatteo et al., 2011a, Agerholm, 2013, Vanderburg et al., 2014, Agerholm et al., 2016, De Biase et al., 2018, Rabaza et al., 2021). *Coxiella burnetii* infections in Cattle describes known clinical manifestations and impacts of C. burnetii infections in large ruminants.

Epidemiology of *C. burnetii* infections in the United Kingdom

In Great Britain, 79.8% and 28.6% of unvaccinated dairy herds (cattle) were found to have the burden of *C. burnetii* infections based on bulk milk tank serology and PCR, respectively (Velasova et al., 2017). It is possible that *C. burnetii* infections may have been negtaively impacting these herds, however, the impact *C. burnetii* infections have on British cattle remains unknown. Changes in legislation (see section Legislation) allow for more frequent investiagtion within British herds, but number of reports are still limited (DEFRA, 2021). The small number of reports may be due, in part, to the lack of inclusion of *C. burentii* as a pathogen during abortion investigations in countries like Scotland (APHA, 2024). Clinical signs are well-reported in large ruminants in countries like France, but intraherd and interherd epidemiology have yet to be investigated in Scottish dairy populations (OSCAR, 2019, Plommet, 1973, Guatteo et al., 2006, Turcotte et al., 2021).

Pathology and clinical manifestation

Q fever – human infections

C. burnetii infections in humans are often asymptomatic (60% of cases), though many (40% of cases) still may suffer symptoms such as pneumonia, fever, malaise and fatigue (Dyer, 1939, Connolly, 1968, Milazzo et al., 2001, Anderson et al., 2005, WHO, 2021, Kersh, 2023). More severe symptoms such as hepatitis (7.4 and 19% of patients with pneumonia or febrile illness, respectively, in Spain), encephalitis and meningitis (1% in France) are also associated with acute forms of the disease (Maurin and Raoult, 1999). Chronic disease may develop with endocarditis and subsequent vascular disease being reported in the majority of chronic Q fever cases (60–70%) (Maurin and Raoult, 1999). Both asymptomatic and symptomatic individuals with Q fever were at risk of developing chronic fatigue syndrome (Derrick, 1937, Connolly, 1968, Spicer, 1978, Penttila, 1998, Milazzo et al., 2001, Schack et al., 2014, Sireci et al., 2021, Kersh, 2023). Disease outbreaks seen in several European, Asian, and South American countries are prime representative cases of the impact Q fever can have on society outside of the agricultural sector (Eldin et al., 2017, Connolly, 1968, Wilson et al., 2010, Vanderburg et al., 2014, Roest, 2011, Beaudeau et al., 2021).

Notable outbreaks

Studies following outbreaks of CAP caused by Q fever investigated what role non-small ruminant farms may have played in the epidemiology of the Netherlands epidemic in 2007. Researchers have identified risk factors for and protective factors against Q fever infections in farm staff that worked on dairy (cattle) farms in the Netherlands during 2010 – 2011. It was found workers had higher likelihood of positivity when staff was recorded as having contact with birth-products, among other factors like contact with pigs, birds present in housing and having a larger herd size (Schimmer et al., 2014). Several protective factors were found including, strict adherence to glove-use during and around calving as well as the use of automated milking systems (Schimmer et al., 2014).

In 2006, Scotland saw its largest outbreak of Q fever amongst workers from a colocated abattoir and cutting plant. The 110 cases were confirmed to originate from sheep lairage, and likely contaminated atmosphere surrounding, and served to set an example for the relevance of Q fever to the public health sector (Wilson et al., 2010).

Diagnosis of *C. burnetii* infections in ruminants

Diagnosis of *C. burnetii* infections in ruminants is often presumptive by ruling out other aetiologies and by a combination of clinical history, using histopathology, qPCR, ELISA, intracellular or axenic culturing methods (OIE, 2018). The price and accessibility of these diagnostics are prohibitive to definitively diagnose *C. burnetii* infections by combining several tests during routine or first-opinion investigation. C*oxiella burnetii* is poorly stained using a simple Gram stain (Derrick, 1937, Burnet, 1941, Maurin and Raoult, 1999, Mori et al., 2017, OIE, 2018). Specialized immunofluorescence antibody (IFA), immunohistochemistry (IHC), Gimenez or modified Ziehl-Neelsen stains are more viable staining techniques used when more sensitive and specific tests, like qPCR, ELISA, or IHC are unavailable.

Historically, histopathology was the most common diagnostic available to veterinarians but conventional molecular diagnostics have become much more cost-effective and accessible as commercial assays have been disseminated into the market (OIE, 2018). According to the World Organization for Animal Health,

due to the lack of specificity of staining as a confirmatory diagnostic, a positive histopathology result can only be used as presumptive evidence of *C. burnetii* infections (OIE, 2018). Furthermore, an additional confirmatory test, such as qPCR, should be performed (Abnave et al., 2017, Ullah et al., 2022, OIE, 2018, Maurin and Raoult, 1999).

Molecular assays used to identify *C. burnetii* DNA within samples like qPCR target genetic segments such as the insertion sequence *IS1111*, found within *C. burnetii* bacterial genome (Roest, 2011, Klee et al., 2006). Diagnostics targeting *IS1111* provides a sensitive, specific and reproducable diagnostic method (Klee et al., 2006, Thomas et al., 2022). Using *IS1111* targeted assays allows for greater detection and subsequent study replication than assays targeting single-gene copy sequences, due to the variable genomic content of different *C. burnetii* strains while providing high diagnostic sensitivity and specificity (Klee et al., 2006, Roest, 2011, Thomas et al., 2022).

Diagnosis can be made difficult if using targeted molecular assays as the only means of diagnosis, as they may not detect all true positive individuals (Abnave et al., 2017, Schramek et al., 1985, Böttcher et al., 2011, Di Domenico et al., 2018). Studies from France investigated the distribution of *IS1111* in *Coxiellia*-like endosymbionts (CLE) (Duron, 2015). The investigation found false positives were attributable to samples containing CLE, such as *Rickestiella spp.* which contain the same molecular diagnostic targets, at levels similar to *C. burnetii* (Duron, 2015).

The Q-Test (CEVA Santé, 2021, France, EU) was created as modified sampling technique which tests for *C. burnetii* via qPCR (Treilles et al., 2021). It aimed to provide a more sensitive and specific test than other diagnostics, like histopathology. These tests were designed for whole herd testing following subfertility or abortions as well as for individual investigation (Treilles et al., 2021). Further investigation may show the potential use for the Q-Test, but combined methodology such as concurrent histopathology, qPCR and/or enzyme-linked immunosorbant assays (ELISAs) remain the main stay of diagnostic investigation of *C. burnetii* infections (Roest, 2011, Böttcher et al., 2011, Guatteo et al., 2011b, Roest et al., 2013, Serrano-Pérez et al., 2015, OIE, 2018).

Coxiella burnetii infections in Cattle

Coxiella burnetii infections have been attributed to three common pathologies, among others, seen in cattle post inoculation during experimental settings and upon post-mortem examination: acute self-resolving pyrexia with proceeding chronic fibrotic pneumonia; bacteremia with associated bacterial valvular endocarditis; and reproductive compromise (Plommet, 1973, Maurin and Raoult, 1999, Martinov, 2008, Böttcher et al., 2011, Agerholm, 2013, Garcia-Ispierto et al., 2014, Schimmer et al., 2014, Agerholm et al., 2016, De Biase et al., 2018, OIE, 2018, Sireci et al., 2021, Bauer et al., 2021). The relationship between *C. burnetii* infections and reproductive disease in dairy cattle is disputed and poorly understood (Agerholm, 2013, Martinov, 2008, Garcia-Ispierto et al., 2014). Previously it was documented that *C. burnetii* was only a potential primary pathogen in cases of stillbirths, premature deliveries and late-stage abortions (Maurin and Raoult, 1999, Agerholm, 2013, Garcia-Ispierto et al., 2014). However, more recent literature has indicated *C. burnetii* as a cause of pregnancy loss,abortion and stillbirths in cattle from fertilization stages to early and late pregnancy loss (Garcia-Ispierto et al., 2015, De Biase et al., 2018, OIE, 2018, Rabaza et al., 2021, OSCAR, 2019). Reproductive diseases that may be associated with *C. burnetii* infections, such as retained placenta, metritis and endometritis, often also follow in cases of twinning, abortion and abnormal or difficult deliveries on Scottish dairy farms, making attribution of a clinical event to *C. burnetii* infections more difficult (Mckay et al., 2023, Turcotte et al., 2021, Agerholm, 2013, Freick et al., 2017, Garcia-Ispierto et al., 2014).

There is no consensus on the definition of *C. burnetii* infections to use when investigating individuals or herds. Many isolates of *C. burnetii*, which have been genotyped using techniques like multispacer sequence typing (MST), have been associated with specific species, regions and virulence factors causing pathogenesis not seen among other isolates, which may explain the lack of agreement on clinical signs of *C. burnetii* infections (Arricau-Bouvery et al., 2006, Agerholm, 2013, Bauer et al., 2021, Truong et al., 2022, Mohabati Mobarez et al., 2022, Sobotta et al., 2022). The varied clinical manifestations from these isolates contribute to the lack of consensus on the impact *C. burnetii* infections have on dairy farms between communities (Rabaza et al., 2021, Epelboin et al., 2023, Arricau-Bouvery et al., 2006, Svraka et al., 2006, Di Domenico et al., 2018). For

example, even non-vaccinated cattle infected with the avirulent Nine Mile strain of *C. burnetii* experience a self-limiting pneumonia and pyrexia (Plommet, 1973). Acute infections have also manifested as a pneumonia that persists (Plommet, 1973, Maurin and Raoult, 1999). *Coxiella burnetii* isolates from cattle induce more acute proinflammatory immune responses *in vitro* compared to sheep or goat isolates, but often less chronic infections *in vivo* (Sobotta et al., 2022).

In France, the second most reported aetiologic agent of cattle abortions is *C. burnetii,* though it is not documented to cause the same rate of abortion in British dairies (OSCAR, 2019). American studies found cows shedding *C. burnetii* via milk to have increased probability of being diagnosed with chronic subclinical mastitis via somatic cell count (SCC) (Barlow et al, 2008). Additionally, the involvement of *C. burnetii* in the Abortion, Premature Delivery, Stillbirth, and Weak offspring (APSW) complex has gained international attention as several compiled studies cited *C. burnetii* infections as an aetiologic agent associated with this syndrome (Ullah et al., 2022, APHA, 2024, Agerholm, 2013). *Coxiella burnetii* has lasting effects beyond pregnancy in both the dam and the offspring (Agerholm, 2013, Radinović et al., 2019, Böttcher et al., 2011). The relationship between bovine *C. burnetii* infections and reproductive diseases (e.g. Metritis, endometritis, pregnancy loss and abortions) warrants further investigation to attribute its relationship to poor production and reproductive health (Martinov, 2008, Agerholm, 2013, Garcia-Ispierto et al., 2014, De Biase et al., 2018, Changoluisa et al., 2019, Rabaza et al., 2021, Turcotte et al., 2021, Ramo et al., 2022, Epelboin et al., 2023). In order to assess the impact of *C. burnetii* infections in cattle, baseline population data, from both the whole herd and potential populations of interest, should be retrieved for analysis and comparison to national averages and herd target KPI. Preliminary studies, such as cross-sectional intraherd investigations are required to begin assembling a clinical and epidemiological profile of *C. burnetii* in Scotland and the UK. A multi-herd study would provide the most insight into the infection status and patterns between herds, as well as highlight agreements or differences between clinical manifestations experienced by respective herd members. Investigations that focus on one herd, rather than many, would still contribute to understanding *C. burnetii* as a pathogen. Single-herd studies would be unable to make assumptions about the behaviour and clinical manifestation of *C. burnetii* infections outside of the herd.

Shedding C. burnetii in cattle

Clinical presentation and detection of shedding vary across studies and examining cows during different life cycle stages. Different routes of shedding provide a variety of options for assessing shedding status of individual cattle, depending on the stage in pregnancy sampling occurs (Guatteo et al., 2006, Guatteo et al., 2007, Barlow et al., 2008, Freick et al., 2017, Szymańska-Czerwińska et al., 2019). Milk, blood, vaginal swabs, and urine can be analysed to determine *C. burnetii* infection status, but sampling using vaginal swabs within seven days following parturition is a suitable method to detect high concentrations of *C. burnetii,* especially when investigating its prevalence in a herd and association with reproductive disease (Guatteo et al., 2006, Guatteo et al., 2007, Guatteo et al., 2011a, Barlow et al., 2008, Agerholm, 2013, Szymańska-Czerwińska et al., 2019, Turcotte et al., 2021). Intraherd inquiries done in other countries yield findings that improve the knowledge of local *C. burnetii* infection epidimiology and pathology, but no equivalent studies have been published for Scotland (Böttcher et al., 2011, Radinović et al., 2019, Patsatzis et al., 2022). Data gathered from a population should be used in conjunction with molecular diagnostics, like qPCR, to reveal potential negative impacts on health, fertility and herd KPI associated with *C. burnetii* infections.

Legislation

In April of 2021 legislation surrounding *C. burnetii* infections changed, making the disease in animals a reportable disease in Great Britain while it remained a category ‘E’ notifiable disease in Northern Ireland. Following Brexit, the UK made changes to the legal requirements for many diseases to ensure trade would continue with the EU as a third country (DEFRA, 2021, DAERA, 2016). *Coxiella burnetii* infections are now receiving a greater degree of national attention. Change to the disease reporting legislation for *C. burnetii* is largely due to it being an adept zoonotic pathogen, and may pose a publich health risk for farmers, veterinarians and the public (Beaudeau et al., 2021, Ullah et al., 2022, Kersh, 2023).

Project objectives

Previous studies on a Scottish commercial dairy farm generated reports that detected increased incidence of uterine infections in cattle and presence of *C.*

burnetii infections in the study farm. Although demonstration of causative associations between clinical outcomes and available diagnostic indicators is challenging, preliminary results on Scottish farms results suggest that *C. burnetii* infections may be contributing to uterine infections and other reproductive diseases including abortion and still births. Assessing the prevalence of *C. burnetii* must be done to begin building a baseline understanding of the bacterial status in the UK.

This investigation aims to:

1 - Quantify the production characteristics and KPI of the study farm

2 – Determine the prevalence of reproductive diseases and define production parameters in the postpartum population

3 – Determine the prevalence of C. *burnetii* shedding in postpartum dairy cows

4 – Quantify the relationships between infection or disease and production metrics in postpartum animals

MATERIALS AND METHODS

Study herd and farm background

The investigation involved a single herd of Holstein dairy cows. The herd was comprised of approximately 1,200 cattle, approximately 900 of which were lactating and 85 of which were dry (not milked) throughout the year. The remainder of the herd, 385 individuals, were classified as youngstock. Cattle on the farm were housed year-round in free-stall cubicle sheds. Groups of heifers were moved five times from weaning up to their first parturition. Off-site housing provided additional space for heifers as well as non-lactating (dry) cows.

The main shed on the dairy farm is comprised of 60 sand cubicles for dry cows, 50 recently calved/late dry cow straw yard and 720 milking cow cubicles. Lactating cows on the farm are milked three times per day at eight-hour intervals. Primiparous and multiparous animals are housed in separate pens. Multiparous cows are further partitioned based on their milk production volume (High-yielding and Mid-yielding). Cows are fed total mixed ration (TMR) based on grass silage, cereals and a concentrate-mineral mix once a day via a single rail feedline barrier, meeting or exceeding the requirements for high-level milk production with ad libitum access to water (NRC, 2001).

The herd has a history of individuals and bulk tank milk samples screening positive for *C. burnetii* by enzyme linked immunosorbent assay (ELISA) and qPCR, respectively. The farm was receiving veterinary consulting service from the Scottish Centre for Production, Animal Health and Food Safety (SCPAHFS), University of Glasgow.

Animal selection

Cows were recruited into the study during weekly visits from SCPAHFS, University of Glasgow veterinary clinicians. Senior farm staff, such as the herd managers, moved postpartum cows into the ‘Postpartum pen’ (holding pen for recently calved cows) following parturition (day 0 of lactation), where they would stay a minimum of seven days. Cows would stay in the postpartum pen longer if veterinary or farm staff observed signs of disease. Cows would leave the postpartum pen prior to the seven-day minimum if they were sold, were moved into another pen by farm staff, had escaped from the postpartum pen

during cleaning or milking or had died. Postpartum cows that calved in the seven days prior to SCPAHFS visits were added to an exam list containing Animal IDs that corresponded with the last four digits of the individuals' ear tag number. The exam list, made by farm staff, was given to the veterinary team for individual examination and sampling of postpartum cows during weekly visits. These individual Animal IDs were then used to identify cows for examination and data collection. Initially, from 13/12/2022–04/04/23, multiparous cows were exclusively examined and sampled. Enrolment of primiparous cows became possible after 05/04/2023 when the examination protocol was expanded to include all lactation/parity groups. Cows enrolled in the study were sampled between one- and seven-days following parturition.

Cows that were listed for examination and eligible for sampling but were absent from the postpartum pen were not enrolled. Intentional removal of cows from the postpartum pen, prior to the full seven days postpartum occurred when there was overstocking in the postpartum pen (≥35 cows). Farm staff would examine the cows that had been in the pen the longest (closest to 7 days postpartum) and move animals without signs of disease to the appropriate pen within the main herd. Cows that were able to escape during the first seven days postpartum into pens rejoined the main herd without sampling and examination.

Herd data acquisition

Individual cow data, KPI and health status for the study were obtained using physical clinical records and the on-farm managerial software (DairyComp305©; Valley Agricultural Software, Tulare, CA, USA). At this farm, data from the Cattle Information service (CIS) were integrated into DairyComp305©, giving variables such as first somatic cell count of the lactation (FTSCC). DairyComp305© also acted as a daily medicine book where farm staff recorded illnesses, medications and treatments indicated by veterinary staff. Individual animal IDs corresponding with these data sets were provided by the software in order to link cows to their KPI and medical history. All variables extracted and evaluated for this study are listed and described in.

DairyComp305© command

The command used to extract variables in from DairyComp305© is:

EVENTS\2SI01 ID DIM AGE LACT BDAT CDAT FDAT DOPN ARDAT CINT CSEX ABDAT PDCC DCC DDRY LST24 TBRD CINT W4MK 1STMK M305 SID SPTAM DID PTBRD FTSCC PTOTF PTOTP PDOPN DINCU EASE %70.13.1 %70.14.1 %70.15.1 %70.32.1 %70.36.1 %70.38.1 %70.3.1 %70.40.1 %70.41.1 %70.42.1 %70.43.1 %70.48.1

Variable handling and combination

Variables extracted from DairyComp305© (**Error! Reference source not found.**) with low numbers of observations (n<12) were excluded or grouped with other physiologically-linked variables considered to be associated with *C. burnetii* infections. The details of the grouping variables created as indicators for "Gestational and obstetric diseases", "Transition disease" and "Exit ≤ 200 DIM" are given at the bottom of Table 1. Individual variables included in these group variables were not also assessed individually in analyses.

Clinical Data Collection

For all enrolled animals DIM, clinical comments and presence/absence of metritis, retained foetal membranes, ketosis and/or hypocalcaemia were recorded. These clinical assessments were recorded and scanned for use as physical health records to import observations into Dairy Comp 305. These observations were exported from Dairy Comp 305 for data analysis. Figure 1 illustrates the timeline relative to sampling when each observation event, including the variables of interest, occurred.

694 *Table 1.* List of all cow level health and demographic variables available for assessment in the study, with information on descriptions and timeline relative to
695 sampling. 'Topic' describes what category of individual, KPI, or clinical data the variable fits within. 'Description' summarises the information recorded for each
696 variable. 'Time relative to sampling' provides the time each event is observed and/or occurs relative to sampling for each cow.

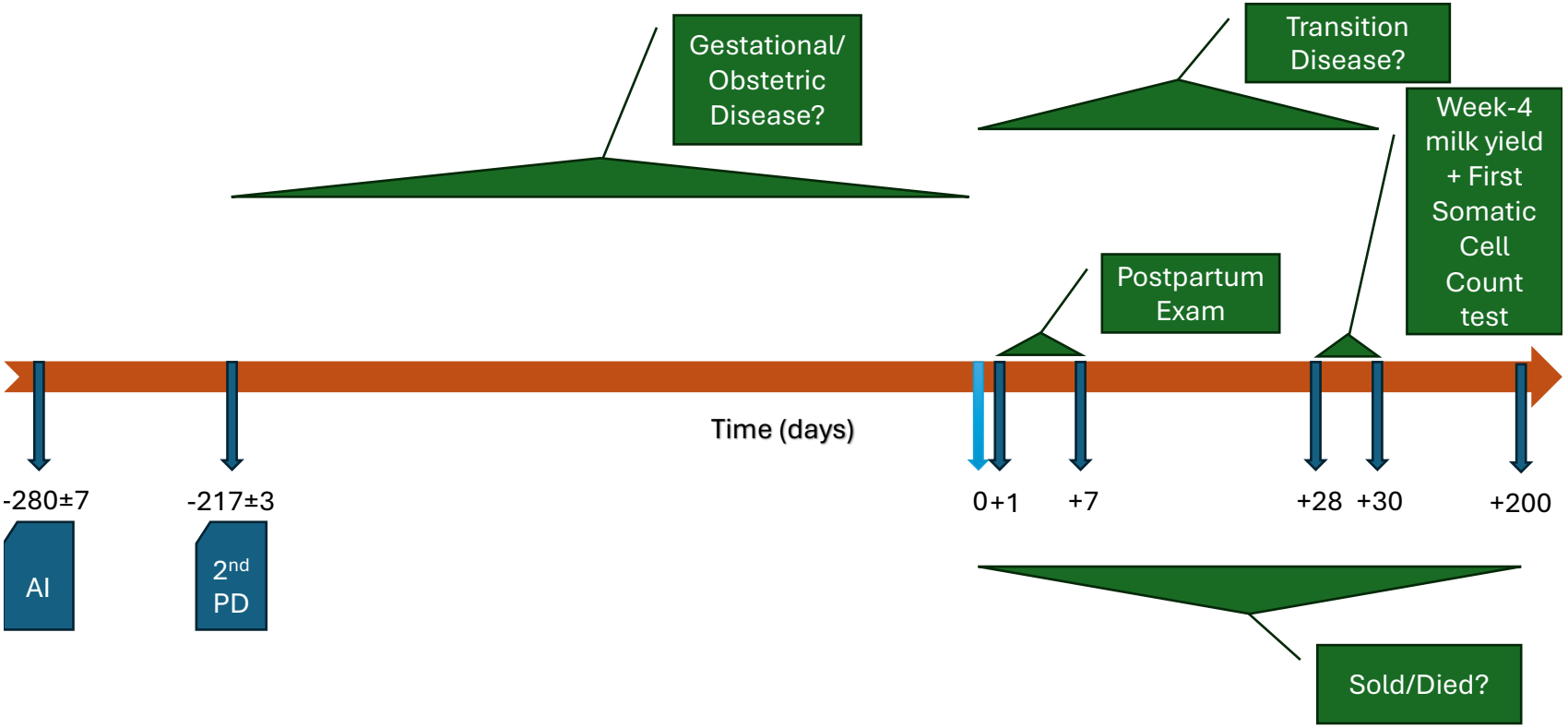
Topic	Variable	Description	Time relative to sampling
Cow attribute	Parity	Number of pregnancies or lactations in lifetime (n+1 after calving)s	New lactation/parity group assigned following most recent calving
	Sire predicted transmissible ability for milk (SPTAM)	Calculated genetic contribution of dam's-sire's ability to produce high volumes of milk	Inherited from dam's sire (grandsire)
Lactation	Days in milk (DIM)	Days since calving and since start of the lactation	1-7 days post-calving (Calving= Day 0)
	Mastitis	Indicator of occurrence or not of inflammatory changes to the teat or milk in ≥1 quarters or FTSCC and/or SCC ≥ 200,000	≤ 90d after calving
	First test somatic cell count (log(FTSCC))	Logarithmic representation/expression of the first somatic-cell-count test of the new lactation	28±2 days following parturition
	Week 4 milk yield (W4MK) (Litres)	Milk yield, 4 weeks postpartum. Representing early lactation yield	Total milk production over first month in current lactation
Reproductive health	Abortion	Indicator of occurrence or not of the loss of viable pregnancy following second ultrasound diagnosed pregnancy	63 ±3 – 270 days following successful AI to calving, prior to sampling

	Premature delivery	Indicator of occurrence or not of the delivery ≤ 271 days in calf	End of gestation, day 0 of lactation
	Prolonged gestation	Indicator of occurrence or not of the delivery ≥ 9 days beyond 279	End of gestation, day 0 of lactation
	Stillbirth	Indicator of occurrence or not of the delivery of dead calf or death of calf ≤ 24 hours after birth	63 ±3 days following successful AI to 24-hours post calving, prior to sampling
	Dystocia	Indicator of occurrence or not of manual assistance during parturition	End of gestation, during parturition, day 0 of lactation
Fertility	Pregnant at first AI	Indicator of occurrence or not of failure to conceive following first service	≤ 280 ±7 days prior to sampling
Transition diseases	Metritis	Indicator of occurrence or not of a uterine infection	Diagnosed following calving and ≤ 21 d postpartum
	Retained foetal membrane	Indicator of occurrence or not of the failure to expel foetal membrane	Diagnosed ≥ 12 hrs postpartum
	Displaced abomasum (DA)	Indicator of occurrence or not of the left or right displacement (+/- volvulus) of abomasal compartment of the stomach	Diagnosed following calving and ≤ 7 d postpartum
	Hypocalcaemia	Indicator of occurrence or not of muscle weakness and/or shaking, cold extremities, nervousness and/or recumbency	Diagnosed following calving and ≤ 7 d postpartum

	Ketonuria	Indicator of occurrence or not of colour change > 'trace' using urine dipstick (KetoStix®, Bayer Diagnostics Europe Ltd., Dublin, Ireland)	Diagnosed following calving and ≤ 7 d postpartum
Combination Variables	Gestational and obstetric disease	Indicator of occurrence or not of ≥ one of the following: Abortion, premature delivery, stillbirth, prolonged gestation, dystocia.	63 ±3 – 270 days following successful AI to calving and/or 1-6 days prior to time of sampling
	Transition disease	Indicator of occurrence or not of ≥ one of the following: Metritis, DA, retained foetal membrane, ketonuria, hypocalcaemia	At time of postpartum examination
	Sold/Died ≤ 200 DIM	Indicator of occurrence or not of ≥ one of the following: Sold ≤ 200 DIM, died ≤ 200 DIM	≤ 200 days postpartum

Figure 1. Visual depiction of timeline of observed events and data capture relative to the timing of enrolling a cow in the study. Green boxes include events of interest, including gestational/obstetric disease, postpartum exam (relative to day=0 of new lactation; days in milk (DIM), Sold/died, four-week milk yield and first somatic cell count test. Dark blue boxes denote significantly timed procedures leading to observed events and sampling.

AI = Artificial insemination
PD = Pregnancy diagnosis



Vaginal Swab Sampling

Vaginal swabs were collected from examined postpartum cows prior to manual vaginal examination. Before sampling, the vulva of the cow was thoroughly cleaned using a solution of Hibiscrub® (Regent Medical Ltd., UK) and then dried with blue roll paper towel (Star Tissue Ltd., UK). The cow's tail was manually lifted while a sterile polystyrene shaft with viscose-tip sampling swab (Technical Service Consultants, TS/19 B) was inserted into the vagina to collect vaginal secretions. Swab tips were cut-off using stainless steel scissors cleaned with 2% Virkon and immediately stored in a sterile 1.2 ml cryovial (Alpha Laboratories, UK) with 1000 µl 1x DNA/RNA Shield® (Zymo Research, Cambridge Biosciences, UK). The collected swabs were stored in a UN37333 compliant travel container until arriving to the laboratory.

Laboratory Methods

Sample inactivation

Upon arrival at the University of Glasgow SBOHVM One Health Research into Bacterial Infectious Disease (OHRBID) laboratory, cryovials containing the swabs, as well as transport container(s), were brought into the MSC II biosafety hood to be cleaned. Sample containers were each wiped down using 2% Virkon. After cleaning, samples were either placed into a -20°C freezer for cold storage or placed into a dry bath (Thermo Scientific FS Isotemp 88860021 Dry Bath Standard Block Heater, Thermo Fisher Scientific, UK). All samples were heat-treated at 70°C for ≥ 60 minutes prior to any further processing to ensure inactivation of *C. burnetii* [Holsinger et al., 2017].

DNA extraction

The DNA was extracted from swab samples using a DNeasy® blood and tissue kit (QIAGEN, UK), following manufacturer's instructions. Briefly, 200 µl swab-cryovial eluent of DNA/RNA® (Zymo Research, Cambridge Biosciences, UK) shield was added to 200 µl AL buffer with 20 µl Proteinase K and vortexed. All tubes were incubated at 56°C for 60-90 min. When the buffer/sample mix had cooled to room temperature, 200 µl ethanol (96–99%) was added, and the tube was vortexed. DNA was extracted from the lysate/ethanol mix. The DNA was incubated in 100µl room temperature nuclease-free water for 120 seconds before Final elution volumes for swab extractions were 100 µl. All batches of nucleic acid extractions (n=10 or 20

samples per batch) included negative extraction controls (n= 2 or 3 controls, respectively) where nuclease-free water was processed during DNA extractions alongside the samples. Sample extracts and negative extraction controls were archived at -20 C for later molecular diagnostics.

Real-time PCR (qPCR)

DNA extracts were tested using an qPCR probe kit (Quantinova, Qiagen) and an IS1111 target sequence assay (Roest, 2011). All samples were tested using one reaction each. All test runs included three dilutions of a commercially supplied positive control with known genome copy number per reaction (100, 1000 and 10000 genomes). No template and extraction controls were also included in every test run. The initial test runs were set to run for 45 cycles, which was reduced to 40 cycles for the rest of samples processed (Figure 3). Samples were considered "negative" if they did not amplify or amplified with a Ct ≥ 40. DNA extracts were then archived at -80 C.

Sample genome copies calculation

Positive controls (1000 genome copies per reaction) that were included in each of the qPCR runs, were used to standardize the Ct values obtained during each qPCR run against a standard curve. For each sample, Cycle threshold (Ct) values and an existing standard curve were used to estimate the number of *C. burnetii* genome copies per reaction using RotorGene Software. For estimation of n *C. burnetii* genome copies per reaction we have assumed that i) the number of copies of *IS1111* in all study samples is the same and ii) the number of copies of *IS1111* in the genome of the positive control is the same as the number of copies of *IS1111* in the genome of all samples tested. To enable modelling analyses of the complete dataset, samples with no amplification by PCR and no observed Ct were assumed to contain a 'very low' concentration of *C. burnetii* and samples that were negative by qPCR were assigned a value of n genomes per sample that was half the lowest value observed in the dataset. The n genome copies per reaction variable was log transformed for model analyses.

Data analyses

All data manipulation and analyses were performed using R (R, 2020). Individual animal level data from dairycomp, clinical observations and qPCR testing were linked for analyses using the unique animal ID. Mixed effect Gaussian generalized

linear models with identity link function (*lmer*) were created to identify variables significantly associated with higher concentrations of bacterial DNA, quantified as log(n *C. burnetii* genome copies per reaction). All models were fit with a PCR run identification variable as the random effect, to account for between-run variation in estimated concentrations of bacterial DNA. The independent variables assessed were chosen to include measures of key production parameters and/or any parameters previously described in *C. burnetii* investigations (E.g., fertility). All variables assessed for significance using *lmer* models are shown in *Table 3*. Univariable models were performed and likelihood ratio tests (LRTs) were used to evaluate significance of all univariable models. All variables with LRT $p < 0.2$ in the univariable model were considered for maximal multivariable model. The initial maximal models were created and then simplified by stepwise removal of variables with LRT $p \geq 0.05$. Variables were removed starting with variables with the largest LRT p value. This process was repeated until all variables retained in the final models had LRT $p < 0.05$. An exception to this process was the handling of the variable DIM which was retained in the multivariable model irrespective of LRT findings, due to previous evidence of a clear relationship between DIM and bacterial load in vaginal swabsEvidence of colinearity between independent variables was assessed via variance inflation factor (VIF) and tolerance calcuation. Independent variables with a VIF value of ≥ 4 and/or a tolerance value of ≤ 0.25 were excluded from the final *lmer* model. The distribution of residuals derived from the final *lmer* model was visually assessed to check normality and assess model assumptions and a Shapiro-Wilk normality test used to detect any deviations from model assumptions. The DHARMa package in R was also used to simulate residuals form the final model to assess evidence that any model assumptions were violated.

Ethics and Compliance

The approval for methods described in this study was given by the University of Glasgow (Research Ethics Committee), license number EA34/22. Detailed informed written consent was obtained from the owner of the farm prior to the beginning of the investigation. PCR positive findings were reported to APHA to comply with UK legislation regarding reportable diseases.

Results

Study herd KPI

Table 2 shows the KPI of the whole herd as they compare to national averages and industry targets for population KPI. The ages of cattle on the farm at the time of the study ranged from 0 days to 144 months of age. The study herd meets or exceeds many of the targets and national averages (Hanks and Kossaibati, 2019). For four metrics, however, the farm data indicates an unwanted deviation form these standards. Whole herd submission rate, defined as the proprotion of cows bred per total cows elligible to be bred, is below the target while the whole herd death rate is higher than the target KPI rates. Additionally, transition diseases, abortions per pregnancy and calf mortality rates rest above target values.

822 Table 2. Study herd and target KPI compared to national KPI averages between 13/12/2022–08/07/2023. Data modelled after SCPAHFS, University of Glasgow
823 monthly herd health report for farm staff and owners. DairyComp 305 (Valley Ag Software, 2023) is used to calculate KPI and herd health parameters against UK
824 targets and averages. Variables where data was available for the sample population (see **Error! Reference source not found.**) were included. Bold variables
825 indicate cases where the study herd KPI positively deviates from KPI target or national average values. Being marked with an asterisk indicate the study herd KPI
826 negatively deviates from KPI target or national average values. Data presented for the study herd represents observations as of 08/08/2023.

827

Key Performance Indicator (KPI)	Study Herd	KPI Target Values[a]	National Average[b]
Herd number/milking	896 (819)	-	214
Milk Yield AV305ME (av L/cow/day)	14,072 (av 41L)	-	8,737 (av 28.6L)
Calvings/year (range per month)	1049 (71-104)	-	-
21-day-Pregnancy Rate	**28%**	**≥ 25%**	**14%**
Submission Rate*	61%*	≥ 70%	39%
Conception Rate	**46%**	**≥ 40%**	**35%**
Cull rate (number of animals/number of calvings)	**27.7% (289/1043)**	**≥ 20%**	**27 %**
Death rate (number of animals/number of)*	5.8% (60/1043)*	< 3%	NA
Somatic Cell Count ('000 cells/mL)	**78**	**< 150**	**171**
Clinical mastitis (cases/100 cows/y)	**15**	**< 30**	**30**
Transition diseases (DA, Ketosis, Milk fever, RP, Metritis)	**10.3% (107/1043)**	**< 10%**	**-**
Abortions per pregnancy per year*	6.3% (46/729)*	<2	-
Perinatal mortality	**3.1% (32/1043)**	**< 5%**	**-**
Youngstock mortality (lact=0)	**4.4% (32/729)**	**< 5%**	-
Calves mortality (<60 d)*	1.5% (11/729)*	< 1%	-

* Whole *herd KPI below target KPI*

[a] *Target KPI (Hanks and Kossaibati, 2019)*

[b] *National averages (Hanks and Kossaibati, 2019)*

[c] *Submission rate: Proportion of cows that are eligible to be bred which are actually bred per heat cycle (21 days)(Hanks and Kossaibati, 2019)*

Summary of sampled population

A total of 324 postpartum cows were sampled between 13/12/2022-08/07/2023. shows the relatinships between the total population available for sampling during the study and the population that was sampled. The ages of postpartum cows sampled during the study ranged from 22-144 months of age. Exclusively multiparous cows were enrolled during these visits until 30/03/2023. Primiparous cows were included in the sampled population beginning 06/04/202 when an updated 'Fresh Check' protocol was implemented to assess their clinical status. Overall, the sampling approach under-sampled primiparous animals (22.1% eligible animals sampled) as compared to multiparous animals (67.1% eligible animals sampled). Furthermore, some cows from these populations were not sampled because they either: Were relocated prior to veterinary checks, died after calving or escaped from the postpartum pen.

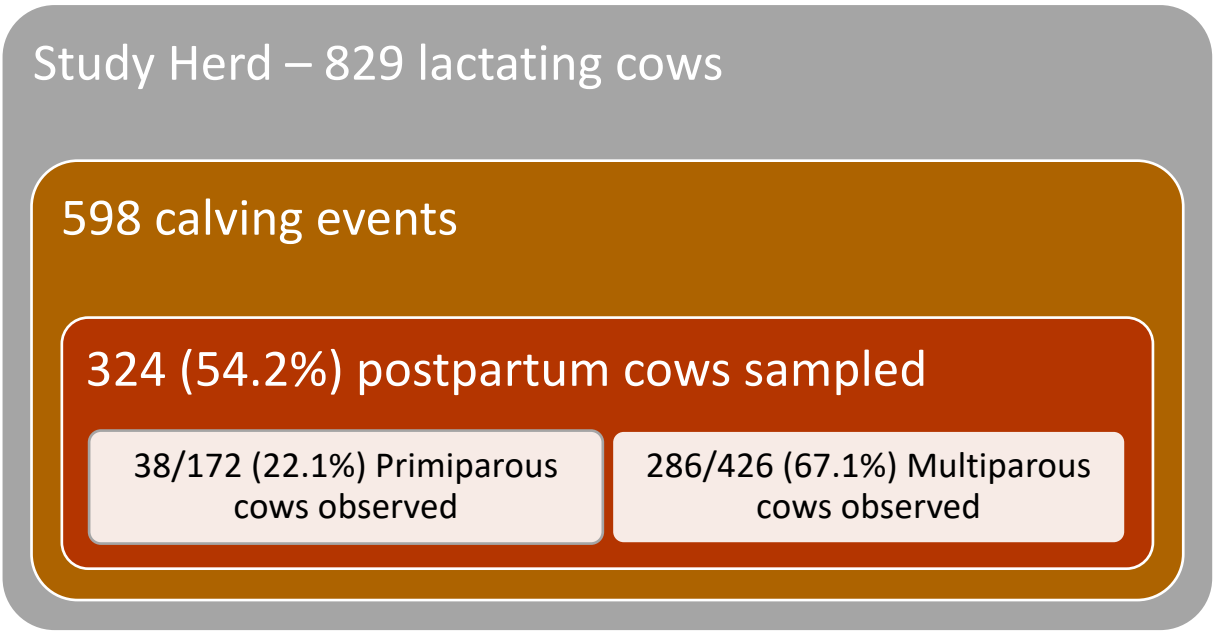


Figure 2. Illustration of the selection process to enrol cattle from the study herd into the sample population between 13/12/2022-08/07/. The study herd averaged 829 lactating cows for the duration of the study. 598 calving events took place during the study period, of which 54.2% (324/598) had a sample taken. Out of the total calvings of multiparous cows, 67.1% (286/426) of the multiparous cows were sampled. Of the total primiparous-calvings during the study period, 22.1% (38/172) of the primiparous cows were sampled.

Coxiella detection in postpartum cows during study period

A total of 324 vaginal swabs were collected from 324 sampled animals. Of the 324 swabs tested, 310 (95.7%) were positive for *C. burnetii* detection based on the observation of amplification and record of a Ct value. All 310 positive samples amplified with a cycle threshold (Ct) value of less than 38.72 in 40 cycles. Results from the qPCR *IS1111* assay showing the calculated log(*n* genomes per reaction) values obtained from the sample population over the period of the study can be found in Figure 3. The n genome per reaction estimated for study samples ranged from 0.0069 to 2,471,818.9. Samples that did not amplify are still shown in Figure *3*, as they were assigned a value of half the lowest n genome copies per reaction. These results were put on a logarithmic scale (elog). Values of log(*n* genomes per reaction) ranged from -5.673–14.720 (Figure 3).

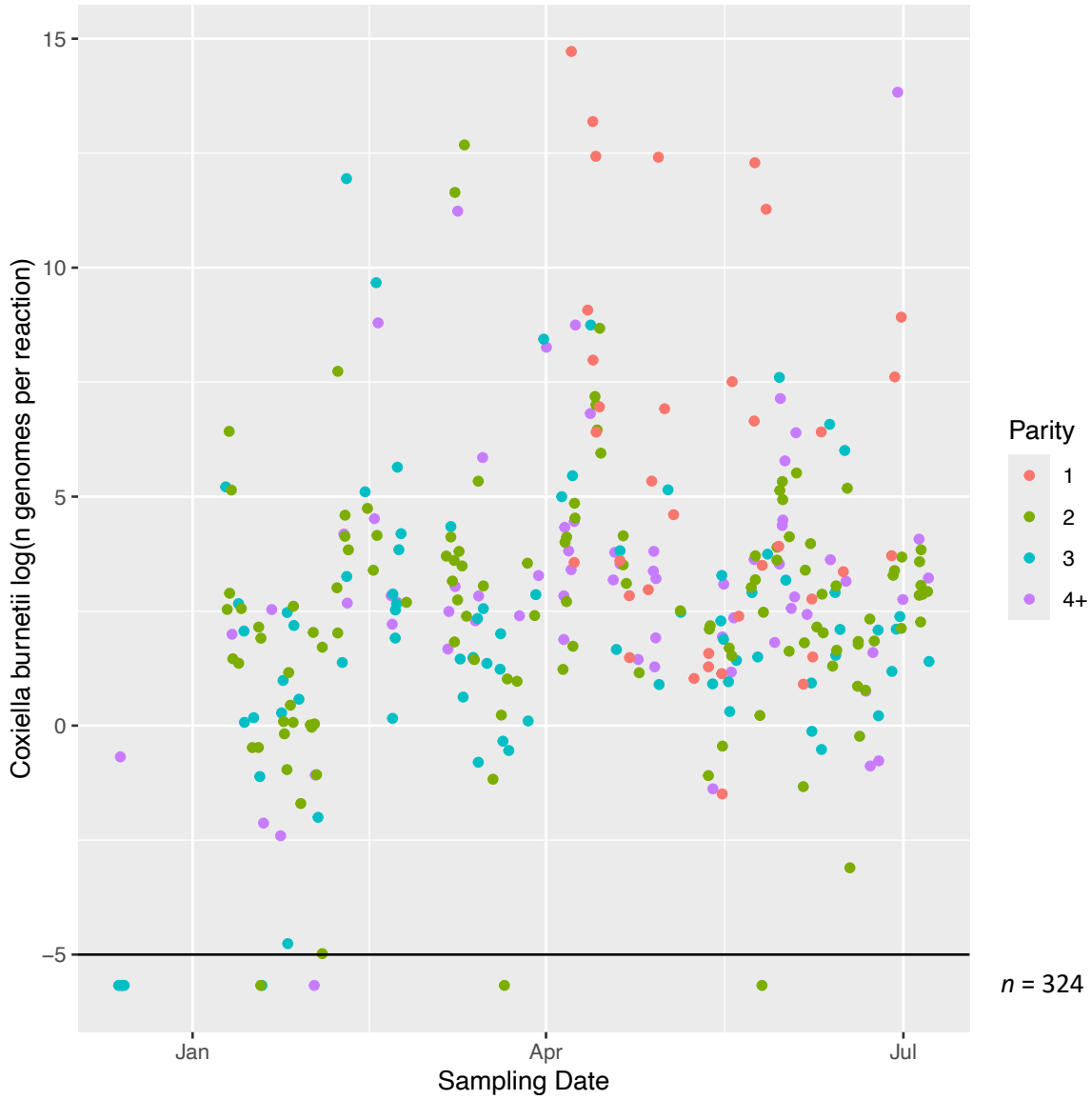


Figure 3. Relationship between *C. burnetii* bacterial load(log(*n* genomes per reaction)) and parity/date sampled in Scottish dairy cattle sampled post-partum (13/12/2022-08/07/2023).

872 Table 3. Summary of the clinical and management characteristics of the study population between 13/12/2022-08/07/2023 while
873 investigating prevalence of *C. burnetii* and shedding in postpartum dairy cows. The sample populations of each variable considered is
874 listed under '*n* Observations'. The sample population total remains n=324. Averages and ranges of log(*n* genomes per reaction) were
875 calculated for both groups of cows that either did ('Yes') or did not ('No') experience one of the disease processes or events. 'Group
876 Variables' included transition diseases, gestational/obstetric diseases or Sold/Died ≤ 200 DIM.

Individual Variable	Level/Average	*n* Observations	Group Variable
Parity		-	N/A
	1	38	
	2	127	
	3	82	
	4+	77	
SPTAM	319.5 (-500 – 860)	324	
Four-week milk yield	47.96 Litres (0 – 156)	324	
Pregnant at first AI		-	
	No	151	
	Yes	173	
Log(FTSCC)	3.183 (0-7.518)	293	
DIM		324	
Metritis		14 = NA	Transition diseases
	No	276	
	Yes	34	
Retained foetal membrane		-	
	No	309	
	Yes	15	
Hypocalcaemia		-	
	No	322	
	Yes	2	

877

Ketonuria	50 = NA	
No	218	
Yes	56	
Displaced abomasum	-	
No	322	
Yes	2	
Dystocia	-	Gestational/Obstetric diseases
No	314	
Yes	10	
Abortion	-	
No	321	
Yes	3	
Stillbirth	-	
No	313	
Yes	11	
Premature delivery	-	
No	312	
Yes	12	
Prolonged gestation	-	
No	312	
Yes	12	
Sold ≤ 200 days postpartum	-	Sold/Died ≤ 200 DIM
No	217	
Yes	107	
Died ≤ 200 days postpartum	-	
No	305	
Yes	19	

Univariable and maximal mixed effect Gaussian generalized linear model building

Univariable mixed effects model analysis with log(*n* genomes per reaction) as the outcome was performed to assess nine independent variables' significance, as shown in **Error! Reference source not found.**. Only one discrete variable was excluded from model analysis, mastitis, due to the failure to meet inclusion requirements *(n* < 12), described in Variable handling and combination. Based on univariable analysis, cows in their first lactation (LRT *P* < 0.001), cows that were sampled fewer days away from parturition (LRT *P* < 0.05), cows that did not become pregnant after first artificial insemination attempt (LRT *P* < 0.05), cows that had lower four-week milk yield (LRT *P* <0.1) and cows diagnosed with gestational and/or obstetric disease(s) (LRT *P* < 0.05) were more likely to have higher concentrations of genome copies per sample (log(*n* genomes per reaction)) based on qPCR analysis. Each of these variables were therefore included in building of a maximal model for multivariable analysis.

Table 4. Summary of all variables assessed in univariable mixed effects linear regression models using log(*n* genomes per reaction) as the outcome. All data were collected from a Scottish dairy herd between 13/12/2022–08/07/2023. Abbreviations: *lmer* = mixed effects linear regression, *p* = *p*-value. Variables that had LRT *P* < 0.2 were included in maximal *lmer* models. If variables were included in maximal model building, variable rows were marked with "yes", whereas variables not included in the maximal model were marked with "no".

Transition disease *n*=274 due to excluded primiparous cows prior to 08/04/2023 and missing data. Four-week milk yield *n*=324 due to death of cow prior to milk production. Log(FTSCC) *n*=293 due to sale/death of cows prior to 30 DIM. The rest of the variable analysis included to whole sample population (*n*=324).

Variables in bold font had LRT *P* value ≤ 0.05 in these univariable analyses.

Variable	Level	*N* observations	log(*n* genomes per reaction)	Univariable mixed effects linear regressions (*lmer*)			Included in maximal multivariable *lmer*
			Mean (Range)	*Estimates*	*CI*	*LRT P*	
Sample population			2.674 (-5.673 – 14.720)				
Lactation group		**324**					**Yes**
	1	**38**	**5.262 (-2.565 – 14.720)**	**-**	**-**	**-**	
	2	**127**	**2.453 (-5.673 – 12.681)**	**-2.46**	**-3.63–(-1.29)**	**<0.001**	
	3	**82**	**1.999 (-5.673 – 11.944)**	**-2.83**	**-4.07–(-1.59)**	**<0.001**	
	4+	**77**	**2.480 (-5.673 – 13.831)**	**-2.39**	**-3.62–(-1.16)**	**<0.001**	
SPTAM		324	-	0.00		0.554	No
Days in milk (DIM)		324	**2.674 (-5.673 – 14.720)**	**-0.18**	**-0.36–0.00**	**0.050**	**Yes**
Four-week milk yield (Litres)		324	-	-0.02	-0.04–0.00	0.082	Yes
Log(FTSCC)		293	3.183 (0.000– 7.518)	0.15		0.332	No
Pregnant at first AI		324				**0.033**	**Yes**
	No	**151**	**2.268 (-5.673 – 11.643)**	**-**			
	Yes	**173**	**3.028 (-5.673 – 14.720)**	**0.75**	**0.06–1.43**		

Transition diseases	274				0.394	No
No	218	2.130 (-5.673 – 11.643)	-			
Yes	56	2.788 (-5.673 – 13.831)	0.32	-0.43–1.08		
Gestational and obstetric diseases	**324**				**0.039**	**Yes**
No	**280**	**2.505 (-5.673 – 14.720)**	**-**			
Yes	**44**	**3.541 (-5.673 – 13.190)**	**1.05**	**0.05-2.05**		
Exit ≤200 days postpartum	324				0.935	No
No	245	2.688 (-5.673 – 14.720)	-			
Yes	79	2.629 (-5.673 – 13.190)	0.04	-0.77–0.84		

Final multivariable mixed effects linear regression model

Multivariable model analysis with log(*n* genomes per reaction) as the outcome was performed to assess variable significance in a mixed effects multivariable linear regression, as shown in **Error! Reference source not found.**. Maximal model simplification excluded variables: Pregnant at first service (LRT $P > 0.05$) and four-week milk yield (LRT $P > 0.05$). The final multivariable mixed effects linear regression model is shown in Table 5. This model, with log*(n* genomes per reaction) as the outcome, included 324 observations, including samples with no amplification on qPCR, assigned half of the lowest detectable value of C. burnetii DNA (log(*n* genomes per reaction) = -5.673). Multivariable analysis determined cows in their first lactation (LRT $P < 0.001$) and cows diagnosed with gestational and/or obstetric disease(s) were more likely to have higher concentrations of genome copies per sample (log(n genomes per reaction)) based on qPCR analysis. Meanwhile, being sampled fewer days away from parturition (LRT $P > 0.05$) had no significant effect on the log*(n* genomes per reaction) within the sample population, according to the final model. No variables in the final model were excluded due to exclusion criteria as described in

Data *analyses*.

Table 5. Variables from assessed for significance using log(*n* genomes per reaction) in a mixed effects linear regression (*lmer*) model in a sample population (*n*=324) from a Scottish dairy herd between 13/12/2022–08/06/2023. Bold indicates variables that were significant with LRT $P < 0.05$, Variance inflation factor < 4 and tolerance < 0.25 in multivariable analyses.

Random effect values: $\sigma^2 = 9.07$, $ICC = 0.15$, $\tau_{00\ Run} = 1.60$, Marginal $R^2 = 0.080$, Conditional $R^2 = 0.218$

Predictors	Level	Final multivariable *lmer*		
N = 324		*Estimates*	*Confidence Interval*	*p*
Random Effects				
(Intercept)		5.31	3.81 – 6.79	<0.001
Lactation group				
	2	**-2.43**	**-3.59 – (-1.27)**	**<0.001**
	3	**-2.73**	**-3.96 – (-1.50)**	**<0.001**
	4+	**-2.32**	**-3.54 – (-1.09)**	**<0.001**
Days in milk (DIM)		-0.14	-0.32 – 0.03	>0.05
Gestational & obstetric diseases		**1.04**	**0.07 – 2.02**	**0.04**

IS1111 qPCR assay: log(*n* genomes per reaction) by parity

The relationship between cow parity and log(*n* genomes per reaction) values obtained are shown in Figure 4. This model, with log(*n* genomes per reaction) as the outcome, included 324 observations, including samples with no amplification on qPCR, assigned half of the lowest detectable value of *C. burnetii* DNA (log(*n* genomes per reaction) = -5.673). The graph indicates higher concentrations of bacterial DNA in primiparous cows as compared to other parity groups. The entire primiparous population had *C. burnetii* DNA detected via qPCR in less than 39 cycles out of 40.

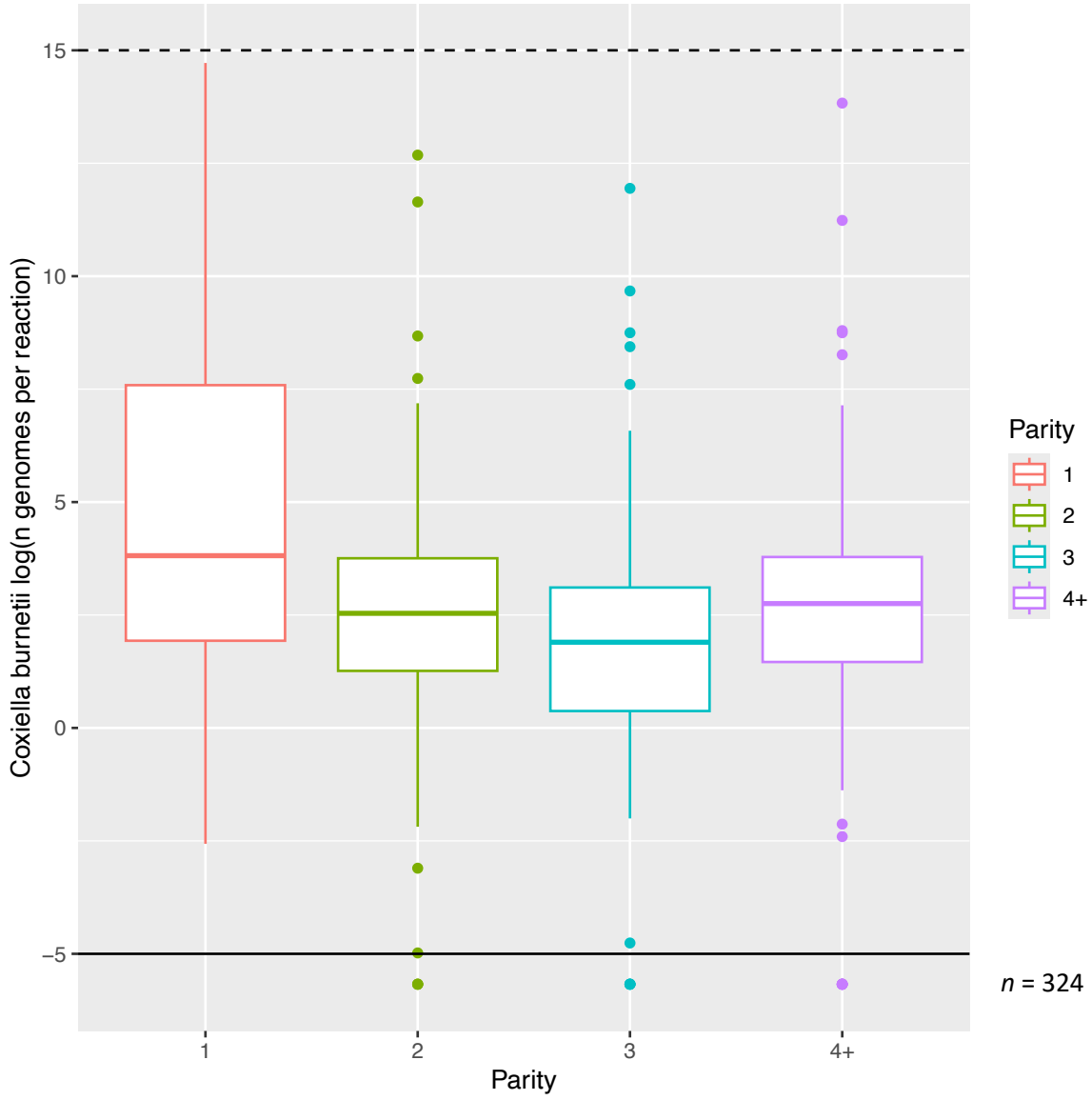
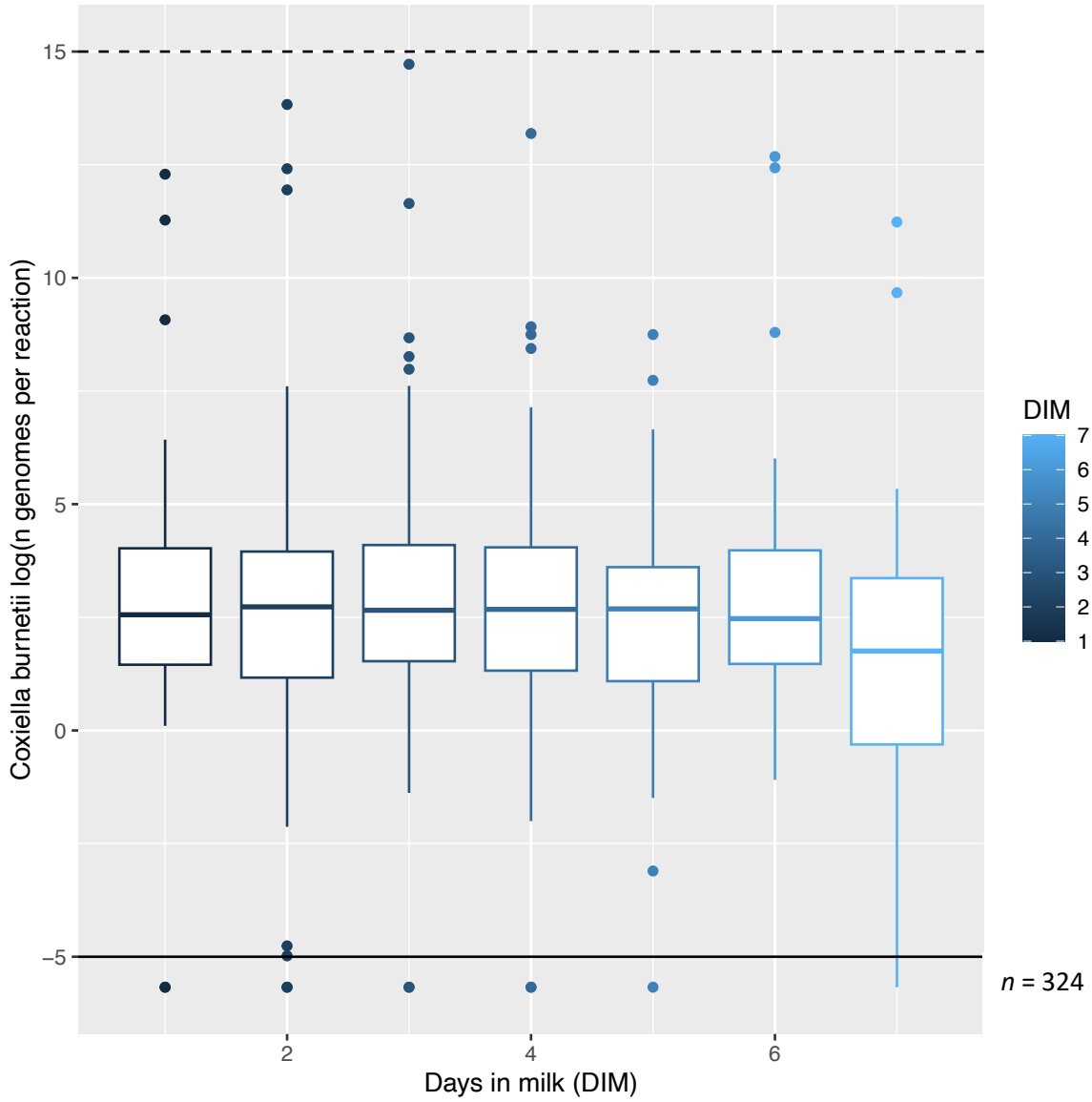


Figure 4. Relationship between parity and *C. burnetii* bacterial load (log(*n* genomes per reaction)) in Scottish dairy cattle sampled post-parturition (13/12/2022-08/07/2023).

IS1111 qPCR assay: log(*n* genomes per reaction) by days in milk (DIM)

The relationship between days postpartum/days in milk (DIM) and log(n genomes per reaction) values obtained are shown in Figure 5**Error! Reference source not found.**. This model, with log(*n* genomes per reaction) as the outcome, included 324 observations, including samples with no amplification on qPCR, assigned half of the lowest detectable value of *C. burnetii* DNA (log(*n* genomes per reaction) = -5.673). The graph indicates lower concentrations of bacterial DNA when cows DIM = 7 cows as compared to cows sampled sooner after calving, but no significant difference was found when assessed using the final mulitivariable model.



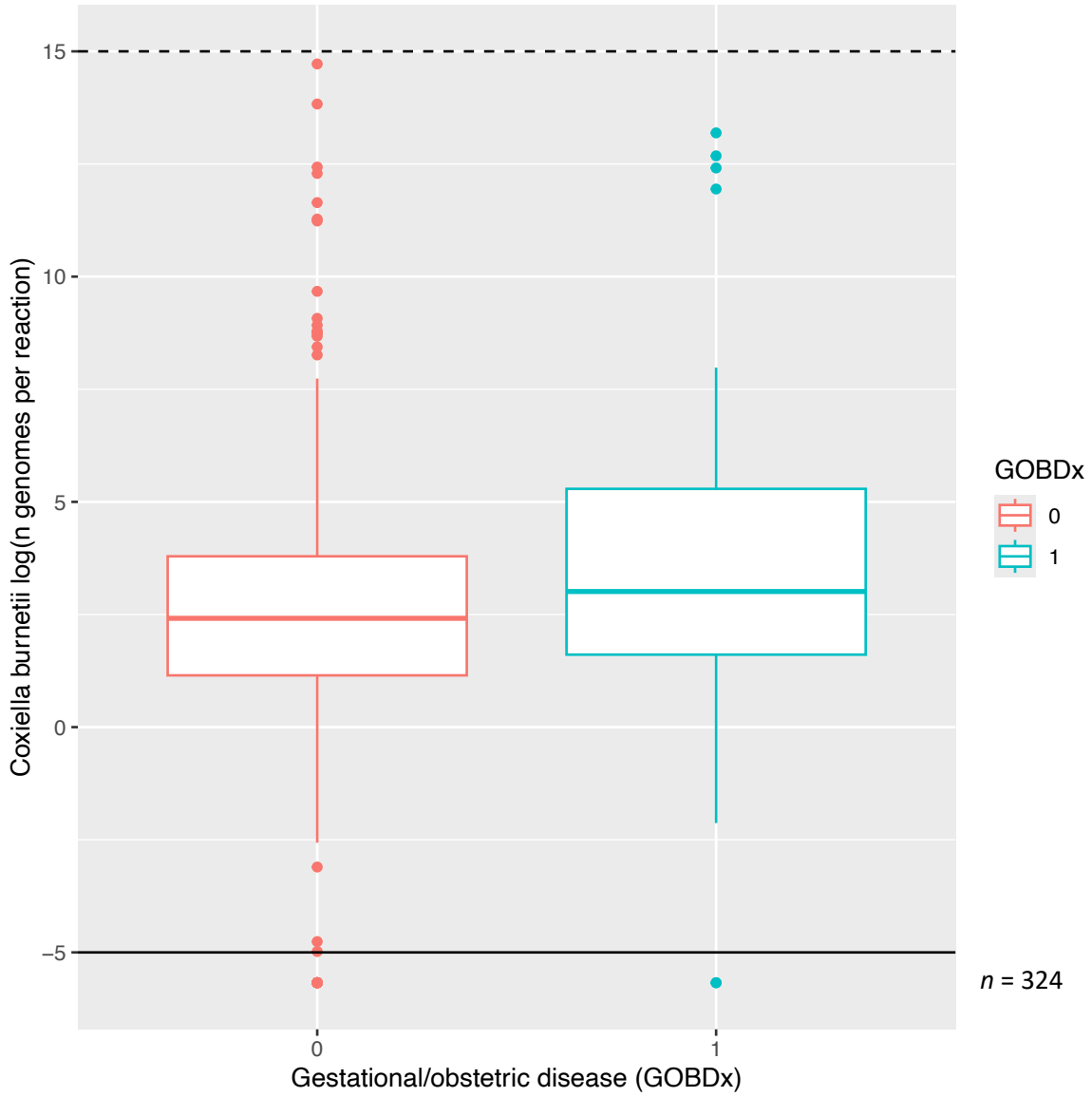
952

953 Figure 5. Relationship between days in milk (DIM) when sampled and *C. burnetii*
954 bacterial load (log(*n* genomes per reaction)) in Scottish dairy cattle sampled post-
955 parturition (13/12/2022-08/07/2023).

956

IS1111 qPCR assay: log(*n* genomes per reaction) by Gestational/obstetric disease

The relationship between being gestational/obstetric disease positive and log(*n* genomes per reaction) values obtained are shown in Figure 6. This model, with log(*n* genomes per reaction) as the outcome, included 324 observations, including samples with no amplification on qPCR, assigned half of the lowest detectable value of *C. burnetii* DNA (log(*n* genomes per reaction) = -5.673). The graph indicates higher concentrations of bacterial DNA were found when cows experienced one or more gestational and/or obstetric disease as compared to cows that experienced none.



968
969

970 Figure 6. Relationship between occurrence of one or more gestational/obstetric
971 diseases and *C. burnetii* bacterial load (log(*n* genomes per reaction)) in Scottish
972 dairy cattle sampled post-parturition (13/12/2022-08/07/2023).

973
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Final mixed effects linear regression model residual

Figure 7 shows an illustration of the resultant estimated marginal means of variables presented in Table 5 after refining the multivariable maximal mixed effects model. This model, with log(n genomes per reaction) as the outcome, included 324 observations, including samples with no amplification on qPCR, assigned half of the lowest detectable value of *C. burnetii* DNA (log(*n* genomes per reaction) = -5.673). Visualization of the predicted values were generated from the final *lmer* model. Predicted log(*n* genomes per reaction) values from the final model and the relationship with the three variables included in the final multivariable linear model analysis are visualized in Figure 7. In the multivariable model, cows that were primiparous (parity=1) and cows diagnosed with ≥ 1 gestational/obstetric disease ((abortion, stillbirth, premature delivery, prolonged gestation and/or dystocia = yes) were found to have higher bacterial loads (higher log(*n* genomes per reaction) values). Primiparous cows diagnosed with ≥ gestational/obstetric disease, on average, had the highest log(*n* genomes per reaction) calculated compared to cows that were multiparous and/or negative for any gestational/obstetric disease. Days in milk (DIM), although included in the final model, was not statistically significant (LRT $P > 0.05$) even though there is gross visual indication of its influence over the outcome.

Distribution of residual values from the model were assessed for deviations from normality. Visual evaluation showed no gross indication of funnelling. Using a Shapiro-Wilk test the study found no deviations from assumptions made by Pearson plot evaluation. Residual diagnostics for mixed regression model detected no problems or patterns in plotting rank transformed model predictions (Residual vs. predicted). Additionally, the q-q plot of residuals dispersion test found no significant deviation when assessing variation of residual distribution.

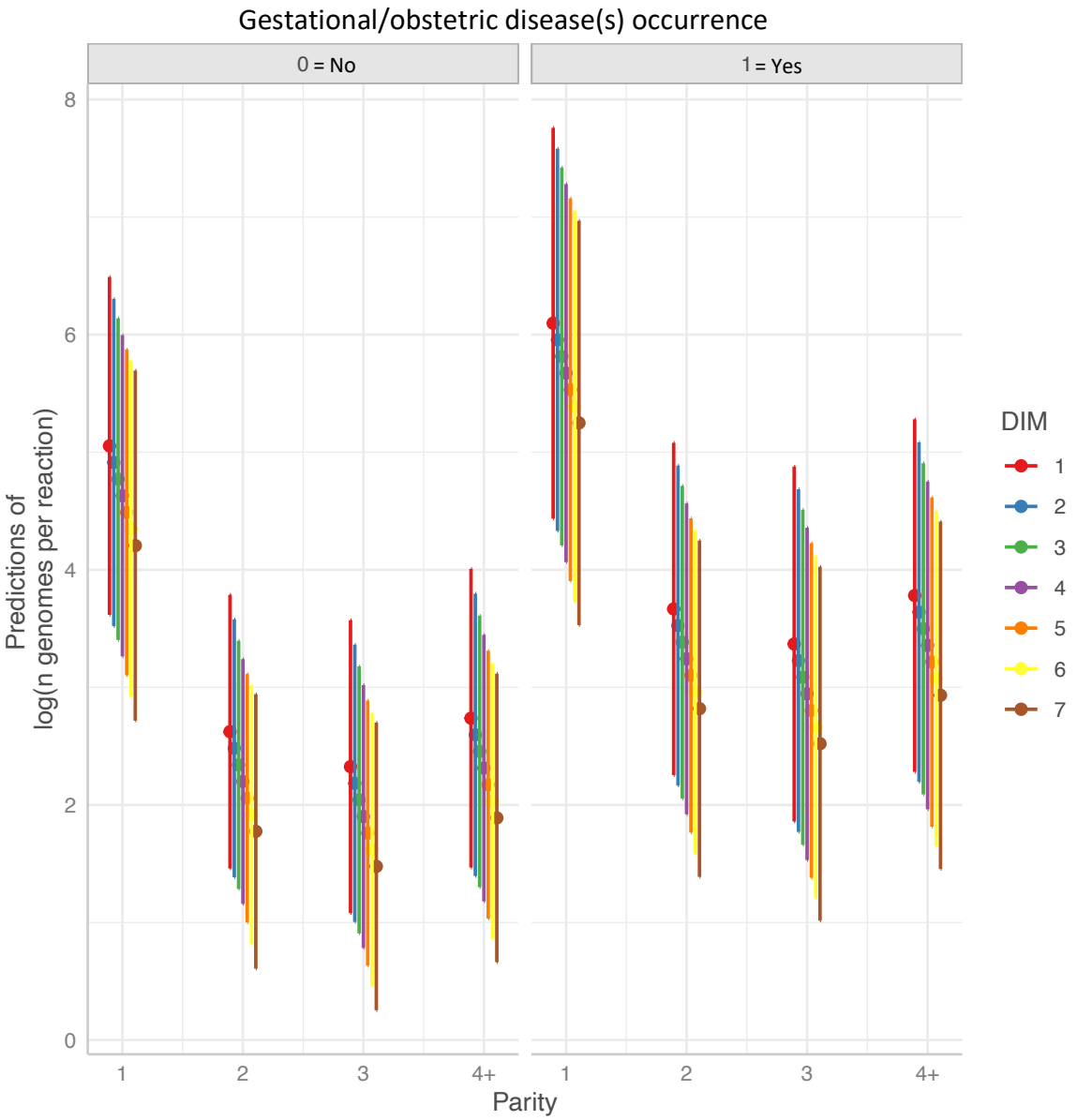

Figure 7. Relationship between adjusted predictions of log(n genomes per reaction) and parity, days in milk (DIM) when sampled and occurrence of one or more gestational/obstetric diseases in final *lmer* model.

Discussion

Coxiella (*C.*) *burnetii* is enzootic to this study herd in Scotland. The herd was found to have extremely high prevalence of cows shedding *C. burnetii* DNA, as well as high concentrations of bacterial shedding in the sampled postpartum cows (Figure 3). Vaginal swab sampling for qPCR analysis, within seven days following parturition, provided effective ability to detect cows shedding various concentrations of *C. burnetii* DNA. Analysis of whole herd key production indicators (KPI) indicate this study herd experienced sub-optimal rates of transition disease, death rate, calf mortality and breeding of eligible heifers and cows compared to KPI targets shown in Table 2. Most of the contribution to increased rates of transition disease found in similar British dairy herds are believed to come from multiparous cows, which this herd is dominated by compared to primiparous cows (Figure 2) (Sheldon et al., 2006, Sheldon et al., 2009, Vazquez Belandria, 2023). This may influence the reported increased rate of transition diseases, as well as increased rate in which cows are sold/culled or die in this herd, as increased parity in dairy cattle is very correlated to rates of diseases.

High producing dairy herds, including the one involved in this study, may lack dramatic presentations of *C. burnetii* infection, such as abortion storms as described in small ruminant herds, but the KPI indicate a harmful prevalence and rate of poorer reproductive outcomes during or following pregnancy, including by means of foetal death at various stages during gestation (Table 2). Molecular detection of *C. burnetii* in cattle has been linked to decreased fertility, reproductive and udder health and production indicating herds, such as this one, may struggle with *C. burnetii* infections (Guatteo et al., 2006, Barlow et al., 2008, Freick et al., 2017, De Biase et al., 2018, Radinović et al., 2019, Guatteo et al., 2011b, Mckay et al., 2023, Sheldon et al., 2006). Though whole-herd KPI meet or exceed nearly all high-end targets for British dairy herds (Table 2), certain KPI, including decreased measures of herd fertility and increased rates of transition disease, death, abortion and calf mortality, indicate a pathology may exist within the herd (Sheldon et al., 2006, Martinov, 2008, Barlow et al., 2008).

The study herd sits above or at most of the national averages/targets which can indicate good management (Sheldon et al., 2006, Sheldon et al., 2009, Vazquez Belandria, 2023). The increased rates of transition or reproductive disease can partly be explained by the age and parities of the population examined. The increased rate of calf mortality and abortions per pregnancy should not be attributable to these same factors (Sheldon et al., 2006, Sheldon et al., 2009, Hanks and Kossaibati, 2019). Failure to reach optimal submission, reproductive disease, perinatal mortality and abortion rates can result from poor or misguided management of herds, as inadequate husbandry and care may result in unsatisfactory production outcomes (Sheldon et al., 2006, Hanks and Kossaibati, 2019). However, Scottish dairy populations which parallel the study population (Table 2) in management strategies, description and location have been enrolled in several investigations which found increased rates of postpartum uterine diseases with several factors associated, including autumn/winter calving and presence of two or more diseases postpartum (first 50 ± 3 days following calving), but did not highlight management strategies as an issue contributing to poor postpartum cow health during uterine health monitoring (Mckay et al., 2023, Vazquez Belandria, 2023, Sheldon et al., 2006). Postpartum populations are in the period of risk for developing most reproductive or transition diseases due to intense environmental and metabolic challenges (Mckay et al., 2023, Sheldon et al., 2006, Hanks and Kossaibati, 2019). Although the initial sampling for this study excluded primiparous cows, this was updated during the study to better assess the whole herd and account for the potential bias created by association between increased parity and the occurrence of diseases during or around parturition.

IS1111 qPCR assays have high diagnostic sensitivity for *C.* burnetii detection due to the presence of multiple copies of IS1111 per genome (Klee et al., 2006). In this study the standard curve produced 100% replication of positives at a concentration of approximately one genome per reaction (1 copy (reaction = 5µl of $2x10^{-1}$ copies/µl) positive control), using the Nine Mile reference strain that has 23 copies of IS1111 per genome. Some studies have raised queries about the specificity of the assay (Duron, 2015). There is evidence to suggest qPCR *IS1111* assays may also detect *Coxiella* like endosymbionts (CLE) (Duron, 2015). This study did not include a comprehensive genetic analysis, so all detections of *C.*

burnetii infections are presumptive (OIE, 2018). Detection of CLE typically occurs in tick species. Studies have not yet confirmed the presence of CLE in samples taken directly form cattle (Duron, 2015). The study farm had a history of being positive via qPCR and serology (for which CLE do not generate false positives) in both individual samples and bulk milk tank screening for *C. burnetii*. Historical results from the qPCR and serology were obtained via an IS1111 assay and ELISA for the detection of *C. burnetii* genome contents and anti- *C. burnetii* (Phase I & II) antibodies, respectively. The historical positive diagnostics and high concentrations of genome copies found in positive samples from results in this study corroborated with the finding of more clinical impact in those with higher concentrations of *C. burnetii* DNA all indicate the bacteria detected in this study are more likely to be *C. burnetii* than CLE (OIE, 2018). Future studies would benefit from using additional *Coxiella burnetii* specific PCR targets such as a *com1* assay, as well as full genomic analysis to confirm the presence of *C. burnetii* (Klee et al., 2006, Duron, 2015, Svraka et al., 2006).

The prevalence of cows shedding of *C. burnetii* in this study population was 95.7% (310/324) and was often shed in high concentrations via vaginal secretion from infected individuals, similar to postpartum cows studied in France (Guatteo et al., 2006). The prevalence of cows shedding of *C. burnetii* in this Scottish dairy farm was much higher than expected. Additionally, the study reported higher rates of bacterial shedding than similar studies from other countries in the UK and mainland European dairy herds were found (Guatteo et al., 2006, Velasova et al., 2017, De Biase et al., 2018). In France, dairy farms have detected shedding via vaginal swab in up to 50% of postpartum herd members sampled (Guatteo et al., 2006, Guatteo et al., 2007). The prevalence of *C. burnetii* infections are particularly high in this population due to the sampling focus on postpartum cows, which have been shown in other studies to have high rates of shedding *C. burnetii* via vaginal secretions during the postpartum period (Guatteo et al., 2006, Guatteo et al., 2007, Guatteo et al., 2011b, Garcia-Ispierto et al., 2014, Piñero et al., 2014, Truong et al., 2022, Patsatzis et al., 2022). Data collected about the prevalence of shedding in postpartum cows indicate the other members of this herd, who may be naïve to *C. burnetii* infections, are at a substantial risk of encountering *C. burnetii* during the

periparturient period. This would also impact animals living in calving pens, where there are high volumes of birth products released into the environment on a daily basis (Maurin and Raoult, 1999, Guatteo et al., 2006).

The study population was biased towards multiparous cows, but it also represents nearly one quarter of the total primiparous population (Figure 2). While the sample population of primiparous cows is smaller, the inclusion of the 'Parity' variable in models accounts for the influence of parity on *Coxiella* shedding alongside the other variables considered (**Error! Reference source not found.**). The study findings suggest that higher *C. burnetii* bacterial loads are associated with increased probability of gestational/obstetric disease outcomes, and that this effect applies across all parity groups (Figure *7*). Further investigation within dairy herds is required to appraise what role *C. burnetii* infections play in poor gestational, obstetric and postpartum health during and after a cow's first pregnancy. Future studies could further assess the health and production impacts cows may experience during their life after detection of high concentrations of *C. burnetii* DNA are found in primiparous cows, as well as any other predispositions that increase the risk of clinical versus subclinical infection manifestations.

Univariable model analysis found significance in several independent variables including parameters contributing to herd fertility, production and clinical and reproductive health. A significant negative association between a cow's four-week milk yield (Litres) and having higher concentrations of bacterial DNA (LRT $P < 0.05$). There is growing evidence in the literature to suggest *C. burnetii* infections have negative associations with mammary health in dairy cattle when shedding detected in milk samples, as well as evidence suggesting mammary-specific pathogenic manifestations, including human milk-borne disease, of *C. burnetii* infections which can correlate to specific MSTs of *C. burnetii* (Barlow et al., 2008, Szymańska-Czerwińska et al., 2019, Rabaza et al., 2021). The results did not provide evidence to suggest the amount of *C. burnetii* being shed had a significant impact on mammary health in this herd. Additionally, cows that were able to conceive a pregnancy after one artificial insemination attempt were found to have a significant negative association with sample concentration of *C. burnetii* DNA in a univariable model (LRT $P < 0.05$). This corroborates well with studies that

reported negative impact on reproductive health and fertility being associated with detection of *C. burnetii* (Martinov, 2008, Agerholm, 2013, De Biase et al., 2018, OSCAR, 2019, Thomas et al., 2022). Neither of these variables were used in the final multivariable model, as both variables lacked significance within the multivariable models (LRT $P > 0.05$).

Multivariable model analysis found significant associations between shedding higher concentrations of *C. burnetii* DNA and parity, with higher bacterial loads shed by primiparous as compared to multiparous cows (LRT $P < 0.001$) and the occurrence (vs not) of one or more gestational/obstetric diseases (abortion stillbirth premature delivery, prolonged gestation, dystocia) (LRT $P < 0.05$). IN contrast to studies from French dairy herds, there was no significant association found between the amount of *C. burnetii* detected by qPCR and the days postpartum,/days in milk (DIM) that each cow was sampled (Guatteo et al., 2007, Guatteo et al., 2011a). These findings further implicates *C. burnetii* as an aetiologic agent involved in the abortion, premature delivery, stillbirth, weak offspring (APSW) complex and reproductive tract diseases in dairy cattle (Martinov, 2008, Agerholm, 2013, Garcia-Ispierto et al., 2014). In several countries, *C. burnetii* infections have been associated with the APSW complex and reproductive tract diseases, such as retained foetal membranes (RFM) (Connolly, 1968, Maurin and Raoult, 1999, Martinov, 2008, Reisberg et al., 2013, Agerholm, 2013). No association was assessed for individual reproductive tract diseases, such as RFM, due to its use as a piece of the group variable 'Transition disease' (Table 3), but all cows diagnosed with RFM, hypocalcaemia and displaced abomasum were positive on qPCR for *C. burnetii* (Table 3). While *C. burnetii* infections continue to be studied further evidence of involvement in poor reproductive health of dams and clinical health of progeny will help fortifying case definitions and clinical profiles. Clear KPI impacts must be understood to inform surveillance, diagnostic endeavours and bolstered control measures for herd and public health.

This study provides a novel dataset to inform prevalence of *C. burnetii* on Scottish dairy farms and further the understanding of impacts *C. burnetii* can have in postpartum dairy cows in the UK. The findings from this investigation identify variables associated with *C. burnetii* infections for cows on this farm

(Figure *6*), as well as parity groups that may be at highest risk for shedding *C. burnetii* (Figure 4). Higher amounts of bacterial DNA being shed was associated with observations of gestational/obstetric diseases in postpartum animals. The range of log(*n* genomes per reaction) values obtained from this sampling strategy highlights key timelines when investigating diseases, like *C. burnetii* infections, that impact reproductive health and production in Scottish dairy herds (Figure *5*). The negative impact *C. burnetii* infections had on postpartum cows during this study were specific to the sample populations' reproductive health, as there was no significant association found between *C. burnetii* DNA loads and other health metrics, like transition disease (**Error! Reference source not found.**). Future work will include molecular characterisation of the bacteria using MST to investigate the potential association of specific genotypes with geographic location and pathogenesis.

Results generated from this study fit well into the clinical picture of *C. burnetii,* but the sampling was restricted to one farm in Scotland over less than one year, so limited conclusions can be drawn about the representativeness of these findings or their relevance for other herds burdened with *C. burnetii* infections. Primiparous cows were not included in postpartum check, and therefore went unsampled, until 06/04/2023, which may have limited the results and conclusions that can be made for cows after their first pregnancy. Visits for sample collection were limited to one time per week, further allowing for there to be cows left unsampled when stocking density became too high in the postpartum pen. The remaining population of cows sampled may have influenced the results of this study, as only cows deemed healthy by herd managers would be moved to make more room ahead of veterinary visits. The removal of the healthiest cows from the postpartum pen ahead of sampling effectively increased the proportion of potentially less healthy cows. The results may then be biased towards unhealthier animals in the herd. This influence farm staff had on the population that was available for enrolment in the study may have introduced bias that affected the findings of the study. There is a lack evidence to suggest an existing genetic predisposition for resistance against disease caused by *C. burnetii* infections, as there are for lameness and mastitis of ruminants (O'Brien, 2017). The scope of this study did not include host genome analysis, although future work would

benefit from investigating how host genetics influences cattle's susceptibility to disease caused by *C. burnetii* infection. Follow-up diagnostic testing, such as histopathology, genomic analysis or ELISAs, were not performed in cases of individual disease to confirm the presence of *C. burnetii* as the aetiologic pathogen. In cases of diseases during the postpartum period, such as RFM or gestational/obstetric diseases, other pathogens should be ruled-out as the aetiologic agent prior to presumptive diagnosis of *C. burnetii* infection as the inciting agent of disease.

This study found negative reproductive health outcomes to be associated with higher load of bacterial shedding, hence these findings present an opportunity to investigate intervention against *C. burnetii* to address negative impacts in the future. In this herd, there was nearly > 95% detection of *C. burnetii*. of the sample population were found with high concentrations of *Coxiella* DNA but less clear immediate impact. These individuals may have a chronic subclinical infection, which is not causing reproductive pathology. Rather, they may experience subclinical infections which allow the animal to continue existing in the herd as an infectious source where it sheds, either intermittently, chronically or sporadically (Guatteo et al., 2006, Guatteo et al., 2007, Guatteo et al., 2011a). It is also possible that these animals are under heavy infectious pressure from this bacterium. In several herds, internationally, detection of *C. burnetii* DNA in milk or vaginal swabs are significantly associated with the development of chronic subclinical mastitis and endometritis, respectively, as well as other obstetric conditions (Martinov, 2008, Barlow et al., 2008). Coxevac has been investigated as a means of reducing clinical burden of *C. burnetii* infections and spread of disease in dairy cattle. Vaccination against Coxiella has shown in select herds to reduce bacterial shedding and improve certain reproductive outcomes, such as abortion rates, outside of Scotland (Garcia-Ispierto et al., 2015, Schulze et al., 2016, CEVA, 2020).

A preliminary study, such as this, provides vital information while building baseline knowledge about an underestimated zoonotic disease that could affect rural and urban Scottish communities and serves as a template for future studies in the UK to assess the impact and burden of *C. burnetii* in dairy herds. With the potential

to spread far beyond the property of this farm, community outbreaks of Q fever should be considered as a potential consequence of such high rates of shedding. Farms like this one should implement basic biosecurity protocols relating to reducing the ability of *C. burnetii* to spread. These may include more rigorous on-farm sanitation protocols, information about zoonotic risk of the MST(s) endemic to the herd remains unknown but should be considered for a herd with such diffuse and heavy shedding of *C. burnetii*. The high shedding rates found within this herd highlights the importance of investigations in British dairy herds and why they should be carried out to better understand the clinical impact on food producing animals, as well as the risk of contracting zoonotic Q fever from cattle.

Conclusion

This high-performing Scottish dairy herd experienced increased rates on transition and reproductive disease and *Coxiella* (*C.*) *burnetii* is endemic to this herd, as qPCR data from vaginal swab samples indicate a high prevalence of shedding (95.7%) via vaginal contents in the herd. This study provided ample ability to detect positive samples and detail associations of negative clinical impact during the postpartum period (Figure 1) in the sample population (Figure 2). A large portion of cows were found to be shedding high concentrations of bacteria (Figure 3), particularly in the primiparous population (Figure 4). In the postpartum sample population cows that were primiparous and/or diagnosed with gestational/obstetric disease(s) were more likely to shed higher amounts of *C. burnetii* DNA (Figure 7) suggesting a harmful influence of higher bacterial concentrations on production and reproductive health. The days in milk (DIM)/days postpartum when sampled did not have a significant influence on the concentration of *C. burnetii* DNA detected (Figure 5). Future work will focus on confirming *C. burnetii* as the pathogen present in these samples using genotyping as a means of investigating the link between MST and impact on KPI and reproductive health.

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