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MS. A. M. HASANTHI ABEYKOON (Orcid ID : 0000-0002-6899-9573)

DR. SIMON FIRESTONE (Orcid ID : 0000-0002-3239-1419)

DR. ANKE WIETHOELTER (Orcid ID : 0000-0002-7872-934X)

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COXIELLA BURNETII IN THE ENVIRONMENT: A SYSTEMATIC REVIEW AND CRITICAL APPRAISAL OF SAMPLING METHODS

Short title: *Coxiella burnetii* environmental sampling

Authors: Hasanthi Abeykoon Abeykoon Mudiyanselage[1], Nicholas Joshua Clark[2], Ricardo Jorge Soares Magalhaes[2,3], Gemma Anne Vincent[4], Mark Anthony Stevenson[1], Simon Matthew Firestone[1], Anke Katrin Wiethoelter[1*]

Institutional affiliations:

1. Faculty of Veterinary and Agricultural Sciences
   The University of Melbourne
   Parkville, VIC, 3010, Australia

2. UQ Spatial Epidemiology Laboratory, School of Veterinary Science
   The University of Queensland
   Gatton, QLD, 4343, Australia

3. Children's Health and Environment Program, UQ Children's Health Research Centre
   The University of Queensland

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South Brisbane QLD, 4106, Australia

4. Australian Rickettsial Reference Laboratory
University Hospital Geelong
Geelong, VIC, 3220, Australia

*Corresponding author, Email: anke.wiethoelter@unimelb.edu.au; Telephone: +61 3 8344 1673

SUMMARY

Q fever is a zoonotic disease caused by the intracellular bacterium, *Coxiella burnetii*. Its primary mode of transmission is by inhalation of aerosols originating from infected animals and contaminated environments. The organism has a very low infective dose, can persist in the environment for long periods of time and large outbreaks fuelled by windborne spread have previously reported. Detection of *C. burnetii* in the environment is therefore important during human and animal outbreak investigations and for the control and prevention of Q fever. This study aimed to systematically review and critically appraise the published literature on sampling methods used to detect *C. burnetii* from different environmental samples. A search of four electronic data bases with subsequent hand searching identified 47 eligible articles published since 1935. These articles described sampling of dust, air, soil and liquids in attempts to detect *C. burnetii* during 19 Q fever outbreaks and in 28 endemic settings. Environmental positivity was most commonly associated with ruminant livestock populations. Evidence describing spatio-temporal characteristics and associated geographical dispersion gradients was limited. The most commonly tested sample type was dust which also yielded the highest bacterial loads of $>10^8$ bacteria/cloth. The MD8 (Sartorius) air sampler was used widely for air sampling. Soil was the only sample type for which a validated laboratory protocol was established specifically for *C. burnetii.* Each environmental sample type has its advantages and limitations which are discussed in detail and a simplified framework to guide decisions around environmental sampling for *C. burnetii* is provided. In any type of environmental sampling, it is recommended to use standardised and validated methods and to match the most ideal sampling strategy and timing with the research context. These conditions are essential to be considered when designing future Q fever management plans that involve environmental sampling for *C. burnetii*.

Keywords

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Coxiella burnetii, environmental exposure, environmental microbiology, epidemiologic methods, Q fever, systematic review

Impacts

- This study presents evidence on environmental sampling methods used to detect *Coxiella burnetii* which is important for environmental assessment of the risk of Q fever from and within livestock holding facilities such as abattoirs and saleyards.
- A variety of sample types was commonly tested, mainly dust, air, soil and liquids in outbreak and endemic settings. Each type comes with its own advantages and limitations.
- Due to the variable presence of *C. burnetii* in the environment in different settings, sampling strategy and timing are important to maximise chances of detection. A simplified framework to guide decisions around environmental sampling for *C. burnetii* is provided.

INTRODUCTION

Q fever is a globally distributed zoonotic disease caused by the intracellular, Gram-negative bacterium, *Coxiella burnetii*. It was first described in 1937 by Edward Derrick as a febrile illness among abattoir workers in Queensland, Australia (Derrick, 1937). Q fever can clinically manifest as acute disease, persistent focalized infection or post-Q fever fatigue syndrome (Ayres et al., 1998; Fenollar et al., 2001; Million & Raoult, 2017). Acute Q fever is characterized by high fever, severe headache, fatigue and chills. The most frequent clinical presentation of the chronic form of the disease is endocarditis. The primary mode of transmission to humans is inhalation of contaminated aerosols. A wide range of hosts including wild and domestic ungulates, birds, companion animals such as dogs and cats, and many species of ticks are known to harbour *C. burnetii* (Woldehiwet, 2004). Infection in domestic ruminants represents the most important infection risk for humans (Georgiev et al., 2013). Bacteria are excreted in large quantities in birth products of infected animals, contaminating the environment. These organisms can become airborne and spread up to 10 km (Clark & Magalhaes, 2018). Economic losses may be experienced by infected farms through increased abortion rates and loss of milk production (Canevari et al., 2018).

C. burnetii has two morphological forms; the large and small-cell variants (LCV and SCV). The LCV is the replicative form (Coleman, Fischer, Howe, Mead, & Heinzen, 2004), whereas the spore-like SCV is metabolically dormant and non-replicative. The SCV is resistant to osmotic and mechanical insults and can remain viable for a long time in the environment (Coleman et al., 2004), enabling *C. burnetii* to infect hosts a considerable distance and time from its deposition at a given source.

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Inhalation of contaminated tick faeces was suggested in early studies as the likely mode of Q fever transmission (Derrick, 1944). Soon thereafter, *C. burnetii* was recovered from air on infected livestock premises and since then, has been detected in other environmental samples such as dust, soil and water. Environmental sampling, in combination with advanced molecular techniques such as real-time PCR, can identify the source of Q fever outbreaks by detecting *C. burnetii* DNA in the environment. This has informed risk assessment and guidelines for Q fever prevention and control (O'Connor, Tribe, & Givney, 2015). Merits and limitations of environmental sampling methods should be considered carefully in order to choose the best sampling strategy for a given context (Lemmen, Häfner, Zolldann, Amedick, & Lutticken, 2001; Thorne, Kiekhaefer, Whitten, & Donham, 1992).

This study systematically reviewed and critically appraised sampling methods used to detect *C. burnetii* in different environmental samples. The aim was to describe and evaluate the environmental sampling strategies employed in terms of sample types, temporal and spatial characteristics of sampling and laboratory methods, with a view to developing recommendations for future environmental sampling and reporting.

METHODS

To ensure transparency, this systematic review was registered with PROSPERO (CRD42018110744) (University of York, 2018) and is reported following the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). The PRISMA checklist on reporting is provided in Appendix 1. The research question was "What are the sampling methods used to detect *Coxiella burnetii* in the environment?". The PICO (population-intervention-comparison-outcome) convention was adopted to formulate the search strategy (Straus, Glasziou, Richardson, & Haynes, 2018): the populations of interest in this study were human and animal populations affected by or with the potential to be affected by *C. burnetii*. The intervention was environmental sampling. Since all environmental sampling methods were assessed, no specific comparison was included in the search strategy. The outcome of interest was the presence or absence of *C. burnetii* in the environmental samples.

A search strategy was initially developed on MEDLINE. The intervention and outcome of interest were used to identify keywords and MeSH headings. Four electronic databases (Web of Science (all databases), CAB Abstracts, Scopus, and MEDLINE) were searched to retrieve peer-reviewed articles published in English. Further articles were identified through e-mail alerts of newly published literature from the databases. Included articles were published between 1935 (the year C. burnetii was discovered) and 2019. Table 1: Comprehensive search strategy for identifying articles shows

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the comprehensive search strategy used. The resulting references from all databases were exported to a reference manager (EndNote X8; Clarivate Analytics) and duplicate records removed.

All articles were initially screened based on their title and abstract. To be included articles must have mentioned undertaking environmental sampling targeting *C. burnetii*. Articles were excluded if they described only mechanisms of windborne spread and did not undertake primary sample collection. Review articles, opinion/commentary or discussion papers, books or book chapters, reports, conference abstracts or proceedings, theses, dissertations, blogs, editorials and newsletters were also excluded. The remaining articles underwent full-text review. References of eligible articles and relevant reviews were hand-searched to identify and include further relevant articles. Data from eligible articles were double entered [by HA and NC] onto pre-defined extraction sheets including epidemiological context (outbreak versus endemic), sampling strategy, detection strategy, detection of viable *C. burnetii*, host species and relevant results. Subsequently, these data were merged, and consensus reached by discussion among the authors on any disparities. Due to the variety of study designs reviewed, no single critical appraisal tool was available that could be used; instead a critical comparison of the relevant study characteristics was produced narratively.

RESULTS

The article selection process is detailed in Figure 1. The search of four databases identified 1839 articles of which 119 duplicates were removed. Screening excluded a further 1651 articles based on the inclusion and exclusion criteria, leaving 69 articles. Hand search and email alerts identified a further 9 articles for full-text review during which 31 articles were deemed ineligible. Thus, data were extracted from 47 eligible articles, with most articles published since 2009 (*n* = 40). Detailed characteristics of the reviewed articles are provided in Appendix 2.

Settings, sample types, geographical locations and host species

The epidemiological context and *C. burnetii* detection success in reviewed articles, is summarised in Table 2. *C. burnetii* was detected in at least one environmental sample type in 42 articles. Among the remaining five articles, one reported inconclusive results (Bond et al., 2016) while four did not detect the organism (Medić et al., 2012; Naranjo, Fustel, Gamarra, Lobato, & Agirre, 2011; Wilson et al., 2010; Woerden et al., 2004). Sample types were predominately categorised as dust, air, soil and liquids. Results are presented separately for these sample types in the following sections. Straw (Karagiannis et al., 2009; Woerden et al., 2004), bedding (Bond et al., 2016), urine, manure and birth products (Bielawska-Drozd et al., 2014) were also tested, but each only in a small number of studies (therefore, not discussed in detail). Dust sampling was conducted most often followed by sampling

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of air, soil and liquids. Two articles failed to provide information on the type of sample (Medić et al., 2012; Wilson et al., 2010). In most articles (*n* = 23) a single sample type was collected, with the most types collected in a single study being four (Bielawska-Drozd et al., 2014). Of the 22 articles that collected multiple sample types, nearly half (*n* = 10) tested a combination of dust and air and all detected *C. burnetii* in at least one sample type.

Studies were most commonly undertaken in the USA (*n* = 10) followed by Spain (*n* = 9), the Netherlands (*n* = 8), France (*n* = 4), Australia, Pakistan and the UK (*n* = 2 each). Other studies were from Belgium, Iraq and Kuwait, Israel, Switzerland, Poland, Iran, Senegal, Japan, Serbia and Italy. Sampling was predominantly associated with humans (*n* = 27) or ruminant livestock (goat *n* = 21, sheep *n* = 16, cattle *n* = 13). Animal species involved and sample types tested in each geographical location with their settings are shown in Figure 2.

Spatial and temporal sampling characteristics

Descriptions of spatio-temporal characteristics and associated geographical dispersion gradients of *C. burnetii* in the environment was limited. As ruminant livestock are likely to pose the greatest infection threat for humans, articles reporting environmental spatio-temporal sampling characteristics related to ruminant livestock (*n* = 28) are summarised in Table 3. Environmental samples were collected from within farm boundaries including milking parlours, animal feeding and holding pens, in 17 articles. Sampling outside farm boundaries were reported for distances from 20 m to 2 km. In most studies where both on-farm and outside farm samples were assessed (*n* = 7), levels of *C. burnetii* DNA were higher on-farm than outside (Álvarez-Alonso et al., 2018; Astobiza et al., 2011a; de Bruin et al., 2013; de Bruin et al., 2012; Hackert et al., 2012). Seventeen articles reported repeated sampling, while eleven articles described one-time sampling.

Dust sampling

Dust in this review refers to dust accumulated over time on a horizontal surface. Twenty-eight articles involved testing dust samples obtained using a variety of methods. Accumulated dust was collected from surfaces by physical removal using either swabs (*n* = 17), sponges (*n* = 2), moistened cloths (*n* = 2), plastic scrapers (*n* = 1), gauze pads (*n* = 1) or vacuuming (*n* = 2). In three studies, swabs or Petri dishes were left in barns to collect newly deposited dust over a 2-week period. Four studies trialled more than one method (Amitai et al., 2010; Joulie et al., 2015; Kersh et al., 2013; Kersh et al., 2010), whereas seven studies did not report the collection method. One article followed a standard protocol for a ready-to-use surface sampling kit using moistened cloths (Carrié et al., 2019). Other descriptions of dust sampling protocols included “swabbing in a single motion over a length of 2

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meters" (Bellini et al., 2014; de Bruin et al., 2011; de Bruin et al., 2013; de Bruin et al., 2012), "wiping 100 cm^2 areas" (Carrié et al., 2019; Joulie et al., 2015), "dragged along the substrate" (Duncan et al., 2013) and "swabs were collected from horizontal surfaces" (Khalili, 2015).

Dust sampling locations differed among articles; some purposefully chose locations to minimize the distance to ruminants and maximize the distance to the main door (Nusinovici, Hoch, Brahim, Joly, & Beaudeau, 2017; Nusinovici, Madouasse, Hoch, Guatteo, & Beaudeau, 2015). Others sampled specific locations such as windowsills, stable boundary walls, fences, feed troughs, ventilation fans and crossbeams within livestock holdings (Bellini et al., 2014; de Bruin et al., 2011; de Bruin et al., 2012; Joulie et al., 2015; Yanase et al., 1998). In settings that did not involve livestock, sampling sites included household or office surfaces (Hurtado et al., 2017; Ratmanov et al., 2013), air conditioning systems (Amitai et al., 2010) and domestic vacuum cleaners (Tozer et al., 2014).

Twelve of the articles that sampled dust were outbreak investigations. Five of these were not associated with ruminant farms; an urban school (Amitai et al., 2010), a waste sorting plant for mixed urban residues (Alonso et al., 2015), a hoist and chain manufacturing plant (Hurtado et al., 2017), a cardboard manufacturing plant (Woerden et al., 2004), and a courier company (Alonso et al., 2019). When comparing the bacterial load in accumulated dust versus newly deposited dust in endemic settings, accumulated dust had higher bacterial counts (1.09×10^8 bacteria/cm^2 of cloth) compared to newly deposited dust (3.3 bacteria/cm^2 of petri dish) (Joulie et al., 2015). However, direct comparisons are difficult due to the differing units.

Air sampling

Articles reporting collection of air, inhalable dust, atmospheric dust and particulate matter with a median aerodynamic diameter of ≤10 µm (PM10) (*n* = 20) are summarised in this section. Of these, 17 described the air sampling device used (see Table 4 for details). These air samplers can be categorized into two groups based on their method of bacterial capture: either a collection liquid (*n* = 3 studies) or a solid collection medium such as a filter (*n* = 13) or cotton plug (*n* = 1). When specified, the collection liquid was either beef extract broth or milk with olive oil. Pore sizes of filters were ≤ 8 µm, with three articles sampling PM10 using filters with 2 µm pores (de Rooij et al., 2016; Hogerwerf et al., 2012; Leski, Malanoski, Gregory, Lin, & Stenger, 2011). The volume of air sampled ranged from 500 to 47,400 L (mode of 500 L), with the largest volume sampled continuously over 79 hours (de Rooij et al., 2016). Height above ground level during sampling varied from 0.3 to 2.5 m. The most commonly used air samplers were the MD8 Airscan or Airport (Sartorius, Germany) (*n* = 7) (Alonso et al., 2019; Alonso et al., 2015; Álvarez-Alonso et al., 2018; Astobiza et al., 2011a; Astobiza et al., 2011b; de Bruin et al., 2013; de Bruin et al., 2012; Hackert et al., 2012).

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Nine articles sampled air during outbreak investigations, with six reporting successful detection of *C. burnetii* and one reporting inconclusive results. The two studies in which *C. burnetii* was not detected were linked to facilities without direct livestock involvement (a waste-sorting plant (Alonso et al., 2015) and a machine-tool factory (Naranjo et al., 2011)), with both sampling within 3 months of the outbreak. All the articles sampling air in endemic settings (*n* = 11) reported detection of the organism. In all studies, the volume of air sampled and height above ground level did not appear associated with the success of detection.

Higher bacterial counts were observed in samples taken during outbreaks compared with those taken one year after the outbreak (de Bruin et al., 2013; de Rooij et al., 2016; Hackert et al., 2012). Bacterial counts were inversely associated with distance from the animal pens/farms (de Rooij et al., 2016; Kersh et al., 2013). *C. burnetii* was detectable, albeit at low levels, as far as 2 km away from farms 8–12 months after abortions or outbreaks (de Rooij et al., 2016; Hackert et al., 2012; Joulie et al., 2015; Kersh et al., 2013). High numbers of goats in the nearest farm and/or less distance between the measurement site and the goat farm was associated with higher odds of a positive air sample (de Rooij et al., 2016). Higher numbers of positive air samples were reported when sampling during periods of higher ruminant activity likely linked to generation of contaminated aerosols (Hogerwerf et al., 2012). Of interest, decreasing temperature, humidity and precipitation appeared to be associated with increased detection of *C. burnetii* in air (de Rooij et al., 2016). One study suggested that the organism attaches to larger dust particles, as *C. burnetii* DNA was detected at twice the rate in inhalable dust (defined as a particle size of up to 100 µm in aerodynamic diameter that enters the mouth and nose during normal breathing and may be deposited in the respiratory tract (Standards Australia Workplace Atmospheres, 2009)) versus PM10 samples (Hogerwerf et al., 2012).

Soil sampling

Twelve articles reported sampling soil, either from the surface or after removing topsoil. The amount of soil sampled ranged from 0.25 (Shabbir et al., 2016) to 200 g (Shabbir et al., 2015), with the majority of studies (*n* = 6) using 5 g for testing (Duncan et al., 2013; Fitzpatrick, Kersh, & Massung, 2010; Kersh et al., 2013; Kersh et al., 2010; Leski et al., 2011; Tozer et al., 2014). Three studies that sampled soil were conducted to determine soil microbiota (Hong, Yannarell, Dai, Ekizoglu, & Mackie, 2013; Leski et al., 2011; Shabbir et al., 2015). The number of soil samples collected was between 20 and 2425 (median = 174).

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From ruminant farms, soil was collected from either within the farm boundary (Bamberg et al., 2007; Kersh et al., 2013; Welsh, Lennette, Abinanti, Winn, & Kaplan, 1959) or outside (Astobiza et al., 2011b). Other studies were associated with non-ruminant species (Duncan et al., 2013; Hong et al., 2013) or sampling to screen large geographical areas (Fitzpatrick et al., 2010; Kersh et al., 2010; Leski et al., 2011; Shabbir et al., 2016; Shabbir et al., 2015; Tozer et al., 2014). Sampling areas in the latter were chosen to represent areas with predominantly human activity (Leski et al., 2011) or with both human and animal activities (Fitzpatrick et al., 2010; Kersh et al., 2010; Shabbir et al., 2016; Shabbir et al., 2015; Tozer et al., 2014). Sample sites within these areas were chosen by convenience or at random.

Sites were sampled either once (Bamberg et al., 2007; Fitzpatrick et al., 2010; Kersh et al., 2010; Leski et al., 2011; Shabbir et al., 2016; Shabbir et al., 2015; Tozer et al., 2014) or repeatedly (Astobiza et al., 2011b; Duncan et al., 2013; Hong et al., 2013; Kersh et al., 2013; Welsh et al., 1959). In endemic settings, sampling was repeated in consecutive lambing seasons (Astobiza et al., 2011b; Welsh et al., 1959). *C. burnetii* DNA was detected in soil up to one year after an outbreak (Bamberg et al., 2007; Kersh et al., 2013)

One out of 2 outbreak investigations and 8 out of 10 articles on endemic settings reported detecting *C. burnetii* or its DNA. It was interesting to note that two different studies, from Australia and Pakistan, using large numbers of samples found similar overall *C. burnetii* prevalence in soil samples of 2% and 1.94%, respectively (Shabbir et al., 2016; Tozer et al., 2014).

Liquid (environmental) sampling

Liquid environmental samples in this review refers to water, sewage and slurry (manure mixed with soil in a liquid form) samples as reported in seven articles. Sources included water from drinking buckets (Karagiannis et al., 2009), standing water on the ground (Welsh et al., 1959), groundwater from the facility and groundwater wells (Hong et al., 2013), an urban river (D'Ugo et al., 2017), sewage tanks of treatment plants (Schets, de Heer, & de Roda Husman, 2013) and slurry tanks in cattle farms (Piñero, Barandika, Hurtado, & García-Pérez, 2014; Piñero, Ruiz-Fons, et al., 2014). The volume of samples tested ranged from 0.5 to 50 L. Most articles did not describe specific sampling methods, however, where reported, samples were collected using sterile evacuated bleeding tubes (Welsh et al., 1959).

On ruminant farms, samples were mainly collected from within the farm boundary (Hong et al., 2013; Karagiannis et al., 2009; Piñero, Barandika, et al., 2014; Piñero, Ruiz-Fons, et al., 2014; Welsh et al., 1959). In other studies, river water was sampled upstream and downstream from a city (D'Ugo

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et al., 2017) or samples were collected from sewage water treatment plants that received wastewater from goat farms (Schets et al., 2013). The frequency of sampling was either once (D'Ugo et al., 2017; Karagiannis et al., 2009), twice (Piñero, Barandika, et al., 2014; Piñero, Ruiz-Fons, et al., 2014) or more (Hong et al., 2013; Schets et al., 2013; Welsh et al., 1959). Sampling was associated with humans (D'Ugo et al., 2017; Karagiannis et al., 2009; Schets et al., 2013), sheep (Welsh et al., 1959), goats (Karagiannis et al., 2009; Schets et al., 2013), cattle (Piñero, Barandika, et al., 2014; Piñero, Ruiz-Fons, et al., 2014) and pigs (Hong et al., 2013).

Two articles (Karagiannis et al., 2009; Schets et al., 2013) reported sampling liquids during outbreak investigations, with positive detection reported in one (Schets et al., 2013). All five articles reporting liquid sampling in endemic settings detected *C. burnetii*. The organism was detected more frequently in samples collected during lambing periods rather than outside of such periods (Schets et al., 2013). Positive slurry samples were reported from farms on which *C. burnetii* antibodies or DNA had been detected in bulk tank milk and at least one animal was seropositive, and in some cases those farms also had positive dust samples (Piñero, Ruiz-Fons, et al., 2014). However, a clear correlation between positivity of slurry and *C. burnetii* seroprevalence in corresponding herds could not be definitively established.

Methods of detection

The most common method used to demonstrate the presence of *C. burnetii* was PCR performed on DNA extracted from environmental samples (*n* = 43). Reviewed articles used a variety of DNA extraction methods and PCR assays (summarized in Appendix 3). The most commonly used targets for PCR were the transposable element IS*1111* and the *com1* gene, either multiplexed (Alonso et al., 2015; de Bruin et al., 2011; de Bruin et al., 2013; de Bruin et al., 2012; de Rooij et al., 2016; Hackert et al., 2012; Hogerwerf et al., 2012; Karagiannis et al., 2009; Schets et al., 2013) or as single assays (Duncan et al., 2013; Kersh et al., 2010; Tozer et al., 2014). Less commonly targeted was the *icd* gene (*n* = 2) (de Bruin et al., 2011; Karagiannis et al., 2009). Among the twelve articles that used more than one target, six categorized their results as “high” and “low” DNA content; detecting only IS*1111* was considered “low”, while detecting both IS*1111* and either *com1* or *icd* implied a “high” DNA content (de Bruin et al., 2011; de Bruin et al., 2013; de Bruin et al., 2012; Hackert et al., 2012; Hogerwerf et al., 2012; Schets et al., 2013).

Quantification was performed in 8 articles, as summarised in Table 5. Of these, 7 used only IS*1111* to quantify bacterial load. Of them, two used standard curves based on known quantities of the *C. burnetii* Nine Mile strain to quantify the experimental strain (Joulie et al., 2015; Kersh et al., 2013). Other articles used commercial kit manufacturer guidelines (Astobiza et al., 2011a; Astobiza et al.,

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2011b; Nusinovici et al., 2017). Dust samples appear to return higher bacterial loads compared to air and soil, however, the results are not directly comparable. Overall, higher bacterial loads were detected from samples taken close to animal birthing areas during or soon after birth.

Preparation of dust, air and liquid samples for DNA extraction was generally achieved by a combination of steps including addition of buffer and release of organisms from the sample matrix aided by a mechanical impulse (vortex/ stomacher/macerating), incubation and centrifugation steps. Given that soil is a more complex substrate than other sample types, recovering *C. burnetii* largely depends on how samples are processed. Six articles reported details of sample processing prior to DNA extraction and PCR, mostly involving steps to remove larger particles either by centrifugation (Duncan et al., 2013; Fitzpatrick et al., 2010; Kersh et al., 2013; Kersh et al., 2010; Tozer et al., 2014) or sieving (Leski et al., 2011). Testing for PCR inhibition was performed in six articles (Astobiza et al., 2011a; Astobiza et al., 2011b; Duncan et al., 2013; Fitzpatrick et al., 2010; Kersh et al., 2013; Kersh et al., 2010). If present, PCR inhibition was overcome by purifying the DNA sample multiple times (Duncan et al., 2013; Fitzpatrick et al., 2010; Kersh et al., 2010), or the sample was excluded from the study (Duncan et al., 2013; Fitzpatrick et al., 2010). Other studies did not specify the outcome if PCR inhibition was detected (Astobiza et al., 2011a; Astobiza et al., 2011b). One article validated a method to extract *C. burnetii* DNA from soil samples (Fitzpatrick et al., 2010), recommending a specific combination of DNA extraction kits. It is important to note that the efficiency of extraction even with these methods was low, with recovery rates in spiked samples ranging from 0.02% to 4.3% (Fitzpatrick et al., 2010).

In addition to PCR, rRNA pyrosequencing and microarray analysis were also carried out using extracted DNA with reported success (Hong et al., 2013; Leski et al., 2011). Several articles genotyped the detected *C. burnetii* strain (Alonso et al., 2019; Alonso et al., 2015; Álvarez-Alonso et al., 2018; Dal Pozzo et al., 2016; Hurtado et al., 2017; Joulie et al., 2015; Kersh et al., 2013), using Multiple Locus Variable number tandem-repeat Analysis (MLVA), Single-Nucleotide Polymorphism (SNP) or Multispacer Sequence Typing (MST).

Eight studies attempted and succeeded in detection of viable *C. burnetii* by inoculation of the samples into laboratory animals (Álvarez-Alonso et al., 2018; Delay, Lennette, & Deome, 1950; Kersh et al., 2013; Kersh et al., 2010; Lennette & Welsh, 1951; Welsh, Lennette, Abinanti, & Winn, 1958; Welsh et al., 1959) or cell culture (Álvarez-Alonso et al., 2018; Bielawska-Drozd et al., 2014; Kersh et al., 2013). These articles are specifically denoted in Appendix 2, which provides further detail. Viable *C. burnetii* was isolated from dust in two outbreak investigations (Álvarez-Alonso et al., 2018; Kersh et al., 2013), air in three endemic settings (Delay et al., 1950; Lennette & Welsh, 1951; Welsh et al.,

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1958) and soil and water during the lambing period in an endemic setting (Welsh et al., 1959). Two articles that demonstrated viable organisms did not clearly state from which sample type the isolates were obtained (Bielawska-Drozd et al., 2014; Kersh et al., 2010). Overall, all viable *C. burnetii* detections were linked to ruminant farms except for one article which sampled a wide geographic distribution (Kersh et al., 2010).

DISCUSSION AND RECOMMENDATIONS

This systematic review examined sampling methods used to detect *C. burnetii* in the environment. *C. burnetii* was detected in dust, air, soil and liquids from a variety of outbreak and endemic settings. Dust was analysed most frequently while air, soil and liquids were sampled in descending frequency, in many countries around the world. Dust was the sample type that demonstrated the highest bacterial load. Detection of *C. burnetii* in a given environmental sample type seems to be related to the source of the organism and the timing of sampling. Limited systematic sampling across spatio-temporal gradients and standardized or validated sampling methods were identified.

C. burnetii was detected in environmental samples mostly in endemic compared to outbreak settings. This could be a result of multiple factors including persistent release of the organism into the environment, sampling and detection methods and the timing of sampling. In endemic settings, all sample types have returned positive results, which indicates that *C. burnetii* is circulating in the air as well as being deposited. Dust, soil and liquids contain particles accumulated over time and thus represent a broader time window in one sample. Most positive air samples in endemic settings were linked to an animal reservoir (ruminants) which is likely due to continuous excretion of the organism into the environment in contaminated birth fluids (Bouvery, Souriau, Lechopier, & Rodolakis, 2003). An outbreak investigation at a waste-sorting plant, on the other hand, detected *C. burnetii* in dust, but not in air (Alonso et al., 2015), despite using the MD8 air sampler, which was successfully used in other studies. The article reported an increase in the volume of waste processed concurrently with the start of the outbreak. This might have led to quicker waste processing meaning that *C. burnetii* was only detectable for a short period in the air, but for longer in dust. In settings with a sustained bacterial source, such as a ruminant farms, the organism is more likely to be detectable in the environment for extended periods (Hackert et al., 2012). However, even in such situations, sampling strategy and timing could have an impact on detection. In an outbreak in an intensive small ruminant farm, air sampling yielded inconclusive results even though animal samples were positive (Bond et al., 2016). Sampling was conducted outside the kidding season and only on a single occasion which might have led to this result (personal communication from authors). Overall, dust, soil and liquids can be used to detect deposited *C. burnetii* over time, and air samples can be

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used if a source is present at the time of sampling to detect circulating *C. burnetii* in air. Obtaining multiple environmental sample types appears advantageous, especially when the source and timing of the release is unknown as at least one type of sample might detect the organism as evidenced.

It is challenging to assess the factors behind non-detection in the four articles. All four of these non-detections sampled either dust, air or did not mention the sample type. Sampling was less intensive than in other studies, with many details on sampling and laboratory methods lacking. They were also outbreak investigations without direct livestock involvement. Meteorological factors such as temperature and humidity have been shown to play a role in detection (de Rooij et al., 2016). If environmental sampling is carried out indoors, weather conditions would not directly influence sampling. However, in the outdoor or indoor climate (cryptoclimate), weather conditions influence particle settling and will consequently impact sampling and detection. Moreover, successful detection is likely associated with proximity to high numbers of animals (de Rooij et al., 2016), which was lacking in these studies. Higher numbers of animals may imply more intensive holdings and more infected animals shedding *C. burnetii*. Therefore, non-detection could be related to a combination of setting, poor choice of sampling strategy and timing of sampling.

Detection of *C. burnetii* DNA does not equate to viability and infectivity of the organism, and therefore bears no direct correlation to infection risk. Performing viability studies for *C. burnetii* is challenging due to the requirement of a Biosafety Level 3 laboratory. All sample types have been shown to harbour viable *C. burnetii,* however, only for a limited period such as after abortion waves/parturition. *C. burnetii* has shown to survive in soil under laboratory conditions for up to 20 days (Evstigneeva, Ul'yanova, & Tarasevich, 2007). In the field, it can remain viable in soil during the lambing period and for one month thereafter.

Collection of dust, soil and liquid samples is restricted by the availability of these substrates. For example, dust samples are present only if there is a surface to accumulate dust. If the sample is standing water on the ground, it might not be reliably available in outbreak situations which are often linked to dry, dusty and windy weather conditions (Hawker et al., 1998; Tissot-Dupont, Amadei, Nezri, & Raoult, 2004). However, collection of dust, soil and liquid samples is more convenient than air sampling due to the minimal need for specialist equipment and therefore suitable for large scale sampling with minimal resources. In contrast, collecting air samples requires a suitable device, which is typically expensive and can be fragile. Further, an air sampler needs to operate for set periods of time to test specific volumes of air, so collecting a large number of samples either requires multiple air samplers used simultaneously or takes long periods of time. The efficiency of the most commonly used apparatus (MD8 Airport/Airscan, Sartorius) has not been

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compared against other air samplers nor against differing concentrations of airborne *C. burnetii* like for other bacterial species and fungi (Bonadonna & Marconi, 1994; Engelhart, Glasmacher, Simon, & Exner, 2007).

It was recently demonstrated that PM10 is a strong risk factor for Q fever in humans (Reedijk, Leuken, & Hoek, 2013). Three articles reported on sampling of PM10 with a 28% and 16% success in demonstrating *C. burnetii* in these samples (de Rooij et al., 2016; Hogerwerf et al., 2012; Leski et al., 2011). However, it should be noted that even with the 2 µm pore size filters used in these studies, capturing the SCV form of *C. burnetii* would be challenging due to its small size (0.2 – 0.5 µm) unless they are attached to larger dust particles.

Soil and slurry samples present difficulties that influence the efficiency of detection due to their complex nature compared to other sample types. The choice of DNA extraction method is therefore important when processing these samples. Among the articles reviewed, the only article that validated DNA extraction kits for processing soil samples for subsequent detection of *C. burnetii* is important to highlight (Fitzpatrick et al., 2010). Most articles published after this followed the same protocol and reported successful results (Hong et al., 2013; Kersh et al., 2013; Kersh et al., 2010; Tozer et al., 2014). Based on the reviewed evidence, this validated method can be recommended for DNA extraction from soil to recover *C. burnetii* DNA. Only one recent article (Carrié et al., 2019) followed a validated method for quantification of *C. burnetii* (Rousset et al., 2012). Other reviewed articles did not perform validations or standardizations of laboratory or sampling methods. Given that *C. burnetii* possesses unique characteristics, only validated environmental sampling methods will accurately indicate the bacterial burden present in the environment.

Decision-making around location and depth of obtaining a soil sample is important and was not extensively discussed in the articles reviewed. Soil from locations where animal carcasses, parturition materials, or contaminated manure or bedding material have been buried or distributed would be more likely to be positive compared to locations in which these activities had not occurred. While choosing sample sites based on convenience in screening studies might be more feasible, those samples are inadequate to assess the overall risk for the general community. Instead random selection of sampling sites within the target area would be desirable and provide more powerful results. Moreover, the type of soil has not been taken into consideration by any of the reviewed articles even though the type of soil may affect the presence of *C. burnetii* (Evstigneeva et al., 2007). Therefore, it is important to report the type of soil together with the results to allow comparison with other studies.

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There was limited opportunity to assess repeatability and reproducibility of methods presented. The level of detail reported on environmental sampling methods, equipment used, and laboratory protocols was sufficient only in some of the articles. Therefore, the validity of the evidence presented in many studies is hard to assess (Bustin et al., 2009; Haddaway & Verhoeven, 2015). Several other articles failed to report the methods followed for sampling processing and DNA extractions. It is recommended that authors explicitly report methods to enable complete evaluation of the evidence and reproducibility of the science.

The multi-copy transposable element IS*1111* was the most common target used for PCR detection and was sometimes used to quantify *C. burnetii* DNA. If this target is used for quantification, the number of organisms present may be overestimated and results will not be accurate unless the exact number of copies present in the sample strain is known (Jones et al., 2011). Recent research demonstrated that IS*1111* was abundantly present in *Coxiella*-like endosymbionts of ticks (Duron, 2015; Jourdain, Duron, Barry, González-Acuña, & Sidi-Boumedine, 2015), therefore the target is not specific to *C. burnetii.* However, the risk of ticks being present in environmental samples is low except for soil. Use of *C. burnetii* specific PCR assays and SNP genotyping methods could ultimately provide confirmation of *C. burnetii* in samples (Pearson, Cocking, Hornstra, & Keim, 2016).

Although this review aimed to capture all relevant published literature, it had several limitations. Only publications available in English were included, which might have left out, for example, some relevant articles from the well-described Q fever outbreak in the Netherlands. Moreover, hand search of reference lists of eligible articles and relevant reviews identified 6 more eligible articles (Figure 1) indicating that the search strategy was not perfect. *Coxiella burnetii* is distributed worldwide (Guatteo, Seegers, Taurel, Joly, & Beaudeau, 2011), however, the geographical distribution of reviewed articles showed that the majority of publications were from Europe and North America with a very limited number of articles from Africa and Asia. This publication bias is well established for other research disciplines (Guerin, Flynn, Brady, & O'Brien, 2009; Sood et al., 2007) and could be due to a number of reasons including language, funding opportunities and divergent animal and health priorities across the globe. Nevertheless, environmental sampling has been predominantly reported related to ruminant livestock. It is therefore not surprising to see more articles published from regions that have large ruminant livestock populations such as North America and Europe (Robinson et al., 2014). In addition, it is well-known that publication is biased towards reporting of positive results (Dickersin, 1997; Rosenthal, 1979). This form of publication bias seems also to be present here, with majority of articles reviewed reporting detecting *C. burnetii* in at

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least one sample (environmental or animal samples). Quantification of these biases is hard to assess, given there is no reliable denominator including, typically unpublished, unsuccessful attempts.

Based on the reviewed evidence, dust, air, soil and liquids are suitable samples for detecting *C. burnetii* in the environment. However, each of these sample type comes with inherent advantages and limitations. Considering the evidence uncovered in this review, a simplified framework to guide decisions around environmental sampling to detect *C. burnetii* is presented in Figure 3. This framework can be helpful in guiding which sample type/s to collect in a given context depending on the suspected source, timing of release and prevailing climatic conditions. This framework is only to be used as a guide as many more context-dependant factors may be important. A single preferred sampling method or sample type for detecting *C. burnetii* in the environment is not identified, instead collecting multiple sample types and reporting detailed sampling and laboratory protocols in future publications is strongly recommended. Reviewed data suggests that there is a need to consider strategic environmental sampling for *C. burnetii* as part of Q fever management plans. This would be particularly important in animal facilities with known history of Q fever and for land surrounding animal facilities which may be developed for human dwellings.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

This systematic review was conceived by MS, SF, GV, HA and AW. HA developed the search strategy, conducted the search, extracted data, summarized and composed the manuscript. NC extracted data. AW guided through the process of the systematic review. All authors contributed to the interpretation of the findings and preparation of the manuscript.

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772

773 **TABLES**

774 ***Table 1: Comprehensive search strategy for identifying articles***

Database	Search strategy	Number of results
MEDLINE	(Coxiella burnetii/ OR Coxiella/ OR Q fever/) AND (exp Environmental Microbiology/ OR "environmental sampling" OR Dust/ OR Soil/ OR Soil Microbiology/ OR water/ OR Water Microbiology/ OR "vacuum sample*" OR Wind/ OR Air Microbiology/)	70
CAB Direct	("environment" OR "environmental exposure" OR "sampling" OR "dust" OR "soil" OR "soil air" OR "water" OR "water microbiology" OR "vacuum" OR "wind" OR "air" OR "air microbiology" OR "air spora") AND ("Q fever" OR "Coxiella	1091

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	burnetii")	
Web of Science	TOPIC: ((Coxiella burnetii/ OR Coxiella/ OR Q fever/)) *AND* TOPIC: ((Environmental Microbiology/ OR "environmental sampling" OR Dust/ OR Soil/ OR Soil Microbiology/ OR water/ OR Water Microbiology/ OR "vacuum sample*" OR Wind/ OR Air Microbiology/)) Refined by: [excluding] DOCUMENT TYPES:(Letter OR Correction OR Book OR Editorial OR Early access OR Reference Material OR Meeting OR Unspecified OR Biography OR Report) AND Languages:(ENGLISH)	617
Scopus	(TITLE-ABS-KEY ((coxiella AND burnetii/ OR coxiella/ OR q AND fever/)) AND TITLE-ABS-KEY ((environmental AND microbiology/ OR "environmental sampling" OR dust/ OR soil/ OR soil AND microbiology/ OR water/ OR water AND microbiology/ OR "vacuum sample*" OR wind/ OR air AND microbiology/))) AND DOCTYPE (ar OR re) AND (LIMIT-TO (LANGUAGE, "English")	61

775

776 ***Table 2 Summary of number of articles that used environmental sample types to detect Coxiella***
777 ***burnetii***

Type of sample	**Number of articles**	**Detected**	**Inconclusive/ Insufficient details provided†**	**Not detected**
Outbreak investigations‡ (n = 19)				
Dust	12	10	1	1
Air	9	6	1	2
Soil	2	1	1	0
Liquids	2	1	0	1
Endemic settings§ (n = 28)				
Dust	16	14	2	0
Air	11	11	0	0
Soil	10	8	2	0
Liquids	5	5	0	0

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[†]Articles presented results of all environmental samples collectively, did not provide results of some sample types or reported results that are inconclusive.

[‡] Q fever outbreak investigations or follow-up sampling after the conclusion of an outbreak.

[§] Vaccine evaluations, environmental investigations on farms, experimental infection studies and studies to detect *C. burnetii* in the environment. [¶]

Some articles tested multiple sample types; therefore the sum of individual sample types in each category are larger than the total displayed.

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785 ***Table 3 Spatial and temporal characteristics of environmental sample types for Coxiella burnetii related to ruminant livestock***

Species	Spatial characteristics			Temporal characteristics		Article
	On-farm/ Within farm boundary only	**Outside farm boundary only**	**Both on-farm and outside of farm**	**Follow-up sampling**	**Frequency**	
Sheep	Yes	No	No	No	N/A	(Carrié et al., 2019; de Bruin et al., 2011; Delay et al., 1950; Khalili, 2015)
	Yes	No	No	Yes	Daily from 1 week prior to parturition until 2 weeks postpartum/ Weekly over 15 months/ Three samplings, one-month apart/ Every 3 and 6 weeks for 9 months after abortions started	(Bellini et al., 2014; Joulie et al., 2015; Welsh et al., 1958; Welsh et al., 1959)
	No	No	Yes No details on the distance of sample collection	Yes	Monthly from start of lambing to end of lactation/ Monthly during lambing period	(Astobiza et al., 2011a; Astobiza et al., 2011b)
	No	No	Yes	No	N/A	(de Bruin et al., 2012)

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			Outside samples: 4 wind directions at 500m radial distances			
	No	No	Yes Outside samples: 4 cardinal directions from farms at 1000 and 2000 m distances/ At radial distances of 500 - 2000 m from farms.	Yes	Monthly from approx. 2 months after the start of outbreak up to approx. 1.5 years/ Outbreak and one-year post outbreak	(de Bruin et al., 2013; Hackert et al., 2012)
	No details provided			Yes	Subsequent kidding seasons after conclusion of outbreak	(Bond et al., 2016)
Goat	Yes	No	No	No	N/A	(Carrié et al., 2019; de Bruin et al., 2011; Karagiannis et al., 2009; Khalili, 2015; Lennette & Welsh, 1951)
	Yes	No	No	Yes	Only in one farm after 11 months	(Kersh et al., 2013)
	No	No	Yes	No	N/A	(de Bruin et al., 2012; Hogerwerf et al.,

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			Outside samples: 4 wind directions at 500m radial distances or upwind and downwind at 20 – 70m distance from farm			2012)
	No	No	Yes Outside samples: 4 cardinal directions from farms at 1000 and 2000 m distances/ At radial distances of 500 - 2000 m from farms.	Yes	Approx. monthly after goat abortions for approx. 1.5 years/ Outbreak and one-year post outbreak/ Monthly up to 7 months	(Álvarez-Alonso et al., 2018; de Bruin et al., 2013; Hackert et al., 2012)

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	No	Yes sewage treatment plants receiving wastewater from farms/ 500 – 1000m buffer zones	No	Yes	Nine samplings during a 5-month period/ Weekly for 28 consecutive weeks	(de Rooij et al., 2016; Schets et al., 2013)
	No details provided			Yes	Subsequent kidding seasons after conclusion of outbreak	(Bond et al., 2016)
	No details provided			Sampling done in two years but in different farms		(Dal Pozzo et al., 2016)
Cattle	Yes	No	No	No	N/A	(Bielawska-Drozd et al., 2014; Carrié et al., 2019; de Bruin et al., 2011; Delay et al., 1950; Yanase et al., 1998)
	Yes	No	No	Yes	Three or two times during a 2-year period; Or Four times over a year or twice during a 2-year period	(Nusinovici et al., 2017; Nusinovici et al., 2015; Piñero, Barandika, et al., 2014; Piñero, Ruiz-Fons, et al., 2014)

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	No details provided	Sampling done in two years but on different farms	(Dal Pozzo et al., 2016)

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788 ***Table 4 Types and characteristics of air samplers used to detect Coxiella burnetii***

Air sampler	**Flow rate/ duration/ volume of air sampled**	**Filter type/ Collection fluid**	**Pore size**	**Sampling level from the ground**	**Article**
Sampling atomizer	45-90 min sampling; 500-900 L	35 mL of beef extract broth at pH 7.2 as collection fluid	NA	Air sampler moved at frequent intervals during sampling	(Delay et al., 1950)
Capillary impinge	0.46±0.01 feet^3/ min at sonic velocity for 60 min; 781 L	30 mL of sterile milk with 2 mL of sterile olive oil as collection fluid	NA	29cm	(Lennette & Welsh, 1951)
Cotton plug sampler	10L/min for ½ - 1 hour; 300 - 600 L	NA	NA	122 cm	(Welsh et al., 1958)
MD8 AirScan (Sartorius)	100L/min for 10 min; 1000 L	Gelatine filter	NS	NS	(Alonso et al., 2015; Álvarez-Alonso et al., 2018; Astobiza et al., 2011a; Astobiza et al., 2011b)

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MD8 AirPort (Sartorius)	50L/min for 10 min; 500 L	Cellulose nitrate filter	8µm	NS	(Alonso et al., 2019; de Bruin et al., 2013; de Bruin et al., 2012; Hackert et al., 2012)
Deployable particulate sampler system	10L/min	47mm, polytetrafluoroethylene (PTFE) filter	2µm	NS	(Leski et al., 2011)
Personal environmental monitor collection heads	4L/min	Teflon filters	2.0µm	150 cm	(Hogerwerf et al., 2012)
GSP collection heads	4L/min	37mm diameter glass fiber filters	NS	150 cm	
SAS super 180	500L of air sampled in 3 min	cellulose acetate and cellulose nitrate filter	0.45µm	NS	(Kersh et al., 2013)
High volume air samplers	NS	glass fibre filter paper	1.6µm	NS	(Tozer et al., 2014)
Coriolis micro	300L/min for 5-10 min; 1500 – 3000 L	NS	NA	30cm above the litter	(Joulie et al., 2015)
Harvard impactors	10L/min for ½ hour for one week; (a 79-hour sample) 47400 L	37-mm Teflon filter	2µm	250 cm	(de Rooij et al., 2016)

789 NA=Not Applicable given the type of air sampler. NS= Not specified in the paper.

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790

791 ***Table 5 Summaries of results from articles that quantified Coxiella burnetii in environmental samples***

Type of sample	Quantity	Location	Species	Time	Article
Dust	1.1×10^8 bacteria /cm^2 of cloth	Barn	Sheep	One month following a primiparous female abortion	(Joulie et al., 2015)
	1 to 3×10^4 bacteria /mL		Cattle	NS	(Nusinovici et al., 2017)
	$>10^8$ bacteria/cloth	NS	Sheep & goat	NS	(Carrié et al., 2019)
	0.3 to 5.8×10^4 bacteria/g (vacuum)	Farmhouse	Goat	During the outbreak	(Kersh et al., 2013)
Air	32.14 bacteria /filter	Inside flock premises	Sheep	On the same month when lambing started	(Astobiza et al., 2011a)
	5.58 bacteria /filter			Two weeks after lambing started	
	12.30 bacteria /filter			One month after lambing started	
	12.94 bacteria /filter			Two months after lambing started	
	5.00 bacteria /filter			During lambing season of the 3rd year after vaccination	(Astobiza et al., 2011b)
	5.42 bacteria /filter	Outside flock premises		On the same month when lambing started	(Astobiza et al., 2011a)
	1.55 bacteria /filter			During lambing season of the 2nd year (coinciding with yearlings' lambing) after vaccination	(Astobiza et al., 2011b)

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	10.59 bacteria /filter			During lambing season of the 3rd year after vaccination	
	<100 bacteria/500 L air	NS	Goat	One-year post outbreak	(Kersh et al., 2013)
Soil/ dust	4 to 4.5 × 10^3 bacteria/g of soil or per swab	Seal resting areas	Northern fur seals	Prior to and during the pupping season	(Duncan et al., 2013)
	6 × 10^3 to 1.4 × 10^6 bacteria/g	Birthing pen	Goat	During the outbreak	(Kersh et al., 2013)
Liquids	30 to > 1 × 10^4 hamster infective doses /mL	Lambing area	Sheep	From the end of lambing to the start of the next lambing season (10 months)	(Welsh et al., 1959)

792 NS= Not specified in the article.

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FIGURE LEGENDS

Figure 1. Flow diagram of the article selection process

Figure 2. Geographical locations and settings of articles that used environmental samples to detect Coxiella burnetii in relation to different sample types(A) and targeted host species (B)

A. Symbols represent dust (), air (), soil () and liquid () sampling in outbreak (red) and endemic (black) settings. B. Symbols represent humans ( ), goats (), sheep (), cattle (), seals ( ) and pigs () sampling in outbreak (red) and endemic (black) settings. They are scaled to represent the number of articles found per country; the smallest size represents 1-2, the middle size 3-4 and the largest size 5-6 articles. Some articles describe multiple sample types and thus are included multiple times.

Figure 3. A simplified framework to guide decisions around environmental sampling to detect Coxiella burnetii

† Requires special equipment to collect samples

‡ Requires complex sample processing techniques with low efficiency

§ Requires a liquid standing on the ground

¶ Requires a surface to accumulate

The climate of a small area, as of a confined space such as a house

APPENDICES

Appendix 1: PRISMA Checklist

Provided as a separate file 'Appendix_1_PRISMA Checklist.pdf'

Appendix 2: Characteristics and results of reviewed articles

Provided as separate file 'Appendix_2_Characteristics and results of reviewed articles.xlsx'

Appendix 3: Details on DNA extraction and PCR assays

Provided as separate file 'Appendix_3_DNA extraction and PCR assays.pdf'

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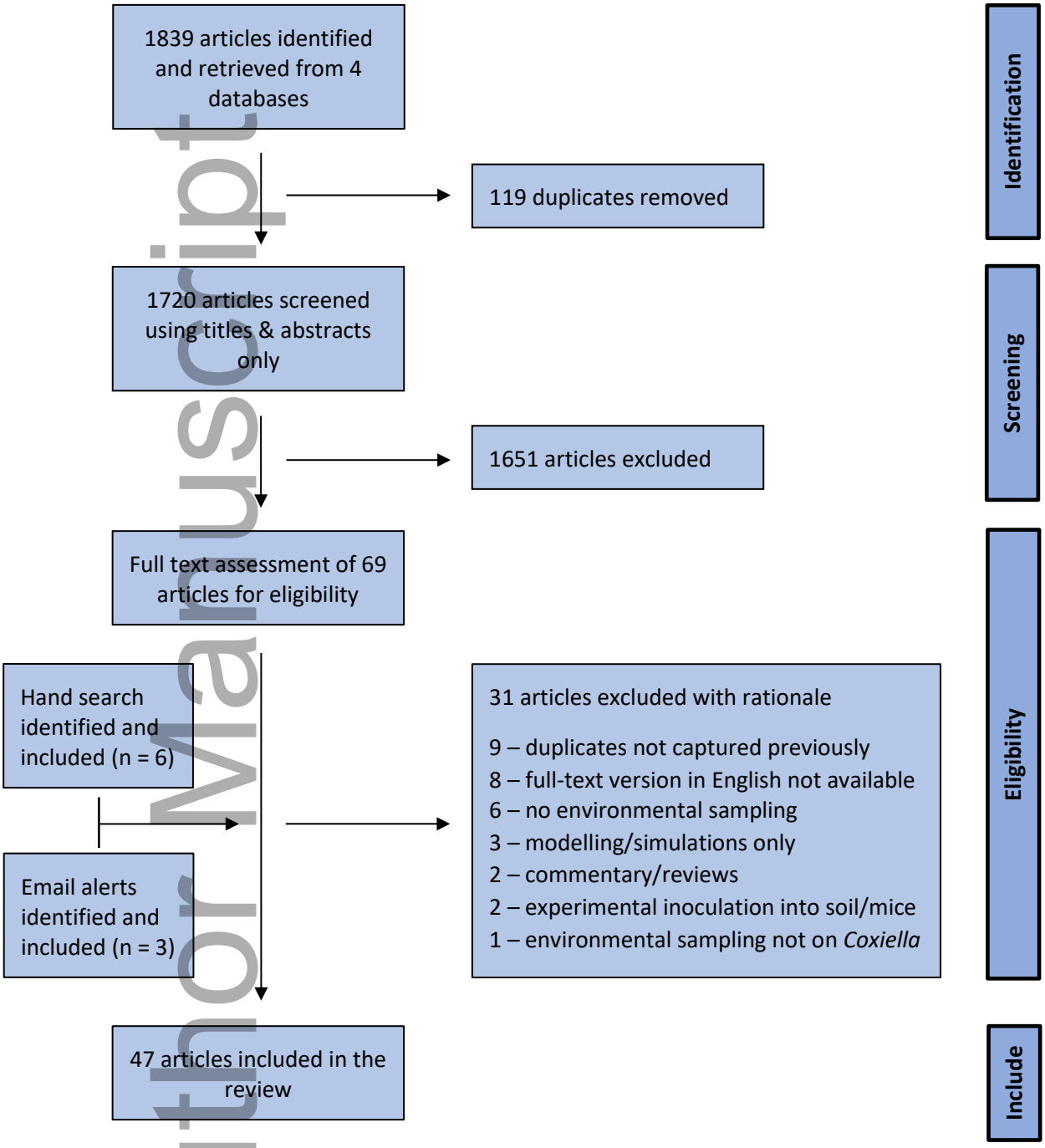

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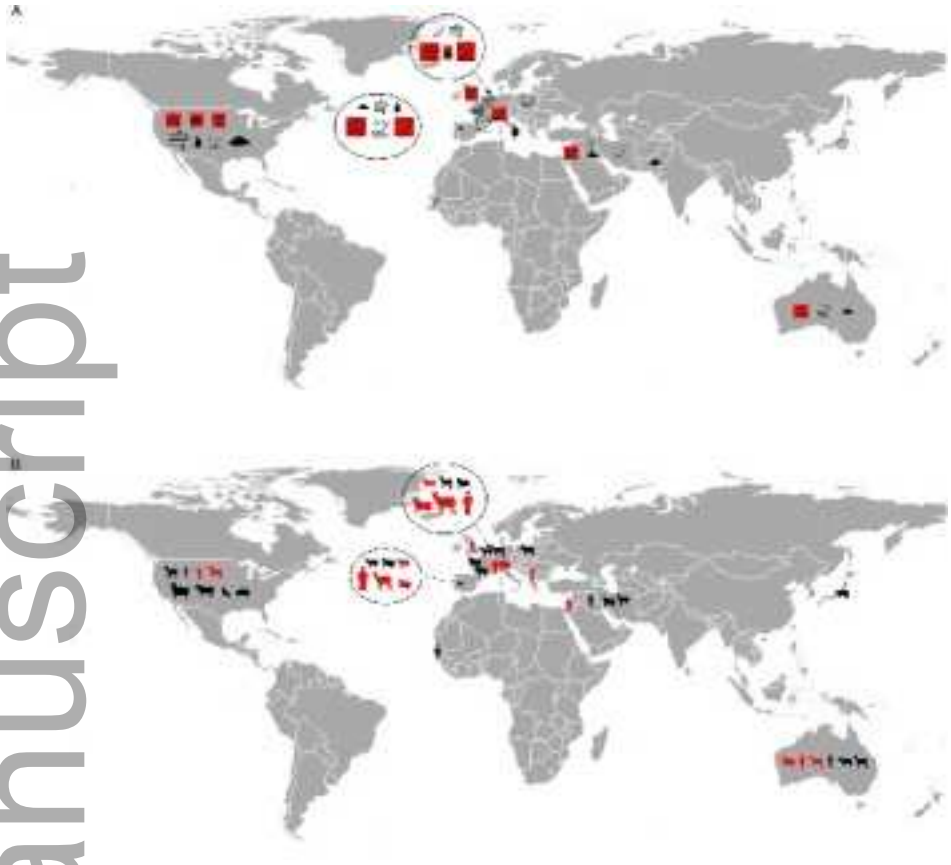

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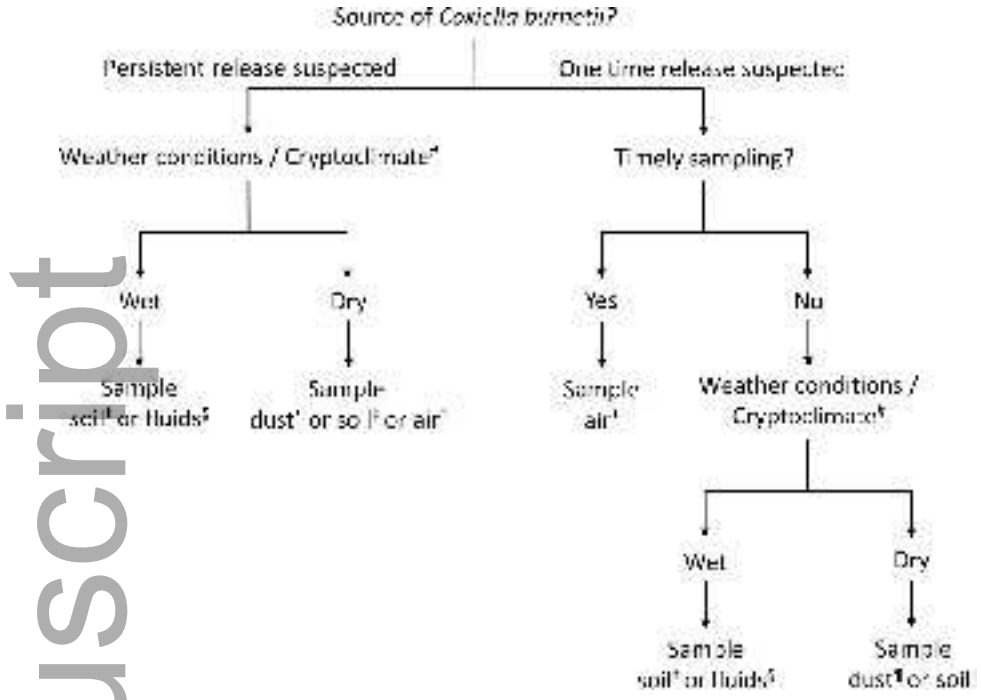

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