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Current perspectives on the transmission of Q fever: Highlighting the need for a systematic molecular approach for a neglected disease in Africa

Running Title: Q fever transmission

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Graphical abstract

Q fever is endemic throughout the world, but remains a largely neglected infection in Africa, where there is a need for systematic molecular epidemiological studies.

Highlights

- Q fever is a bacterial zoonotic disease
- Acquired mainly from livestock
- The infection is endemic in Africa but studies are largely restricted to serology

- Analysis of bacterial genotype is key to understanding transmission and pathogenesis
- There is a need for systematic molecular epidemiological studies in Africa

Abstract

Q fever is a bacterial worldwide zoonosis (except New Zealand) caused by the Gram-negative obligate intracellular bacterium *Coxiella burnetii* (*C. burnetii*). The bacterium has a large host range including arthropods, wildlife and companion animals and is frequently identified in human and livestock populations. In humans, the disease can occur as either a clinically acute or chronic aetiology, affecting mainly the lungs and liver in the acute disease, and heart valves when chronic. In livestock, Q fever is mainly asymptomatic; however, the infection can cause abortion, and the organism is shed in large quantities, where it can infect other livestock and humans. The presence of Q fever in Africa has been known for over 60 years, however while our knowledge of the transmission routes and risk of disease have been well established in many parts of the world, there is a significant paucity of knowledge across the African continent, where it remains a neglected zoonosis. Our limited knowledge of the disease across the African sub-continent have relied largely upon observational (sero) prevalence studies with limited focus on the molecular epidemiology of the disease. This review highlights the need for systematic studies to understand the routes of *C. burnetii* infection, and understand the disease burden and risk factors for clinical Q fever in both humans and livestock. With such knowledge gaps filled, the African continent could stand a better chance of eradicating Q fever through formulation and implementation of effective public health interventions.

Keywords

Coxiella burnetii; Q fever; Zoonosis; Livestock; Africa

1. Introduction

Q fever is a zoonotic disease with a worldwide distribution except for New Zealand and Antarctica. The first identification of Q fever or 'Query fever' was published in 1937 (Derrick, 1937). However, Q fever remained a relatively neglected disease, until the recent national outbreak in the Netherlands between 2007 and 2010, which demonstrated the potential of the disease to be a major public health issue (Schneeberger et al., 2014).

The causative agent of Q fever is the bacterium *Coxiella burnetii, a category B* bioterrorism agent. *C. burnetii* has a wide host range including humans, ruminants (goats, sheep, and cattle), as well as companion animals and ticks. In livestock, *C. burnetii* infection remains often clinically inapparent, except in cases of abortion, particularly in goats and sheep (Van den Brom et al., 2015b). In humans, infection can cause an acute form of disease, which typically exhibits itself as pneumonia and a chronic form resulting in endocarditis, which is potentially fatal (Gikas et al., 2010).

In recent years, our understanding of the transmission of Q fever has been transformed by the use of molecular typing techniques. However, our knowledge has remained largely defined by studies in Europe and North America. The presence of Q fever in Africa has been recognised for over 60 years (Kaplan and Bertagna, 1955), however it remains a relatively neglected zoonosis across the continent, and our knowledge of the disease across the continent is largely through serology and sero-epidemiological studies. The purpose of this review is to provide a general overview of the Q fever bacteriology, pathology, and infection of relevant host, reservoirs and transmission as well as existing genotyping techniques. Most significantly, a special focus is given to revealing the lack of systematic and comprehensive studies of the molecular epidemiology of Q fever in Africa.

2. Clinical Q fever

2.1 Human infection

Clinical manifestation of Q fever occurs as either an acute or a chronic form of disease. Approximately 40 % of infected people will develop symptomatic acute Q fever of which, the majority will present as a non-specific, self-limiting illness. More severe clinical symptoms include fever, headache, chills, atypical pneumonia and hepatitis, (Derrick, 1973; Maurin and Raoult, 1999; Raoult et al., 2005). Acute episodes are rarely fatal unless the patients also suffered from severe underlying medical conditions (Kampschreur et al., 2010). Approximately 20 % of acute Q fever sufferers experience symptoms of long-term fatigue, which frequently lasts beyond a year and more commonly between 5 to 10 years after the initial episode. This is termed post Q fever fatigue syndrome (QFS) and while the symptoms are poorly defined, sufferers experience significant long-term impaired health status and reduced quality of life (Morroy et al., 2016). Chronic Q fever develops in less than 5 % of patients in which symptoms may only manifest years after the initial infection. Clinical symptoms include non-specific fatigue, fever, weight loss, night sweats and hepato-splenomegaly as well as endocarditis (Raoult et al., 2005; Wegdam-Blans et al., 2012). After the recent outbreak in the Netherlands, vascular complications appeared to be more common than endocarditis, however due to the relatively short period of time since the outbreak and the slow clinical progression of disease, endocarditis may not have become apparent yet in many chronically infected individuals (van der Hoek et al., 2012). Chronic Q fever has a wider clinical presentation and has recently been recommended to undergo a nosological refinement to accommodate all probable manifestations, for a better clinical diagnosis (Eldin et al., 2017).

2.2 The role of livestock in the transmission of Q fever

While *C. burnetii* infects a number of animal species, the majority of human infections are thought to be associated with livestock, although infection through contact with wildlife (kangaroos, wallabies and three-toed sloth) (Davoust et al., 2014; Eldin et al., 2015; Maurin and Raoult, 1999), and companion animals (Buhariwalla et al., 1996; Komiya et al., 2003; Kopecny et al., 2013; Kosatsky, 1984; Langley et al., 1988; Laughlin et al., 1991; Malo et al., 2018; Marrie et al., 1988; Pinsky et al., 1991), have been reported. In Infected livestock, the bacteria may be shed in significant quantities in birth products, vaginal mucus, urine, faeces, or milk. The bacterium is highly infectious (1-10 bacteria) therefore this high bacterial load in birth products is a potent source of infection.

In non-pregnant livestock, *C. burnetii* infection is virtually asymptomatic. The most important clinical presentations of Q fever in livestock species are reproductive anomalies such as, abortion, stillbirth, infertility, endometritis, and mastitis. Q fever primarily causes abortion in goats, sheep and less commonly in cattle. The highest concentrations of *C. burnetii* (up to 10^9 organisms per gram of tissue) are found in the placentas of infected livestock, either after abortion or indeed after livebirth (Berri et al., 2001; Guatteo et al., 2011; Hatchette et al., 2003; Lang et al., 1991; Rousset et al., 2009; van Moll et al., 1993). Abortion occurs most frequently at the end of gestation, without any preceding clinical symptoms (Arricau-Bouvery et al., 2005). In goats, abortion, stillbirth but also the birth of strong and lively kids can occur after Q fever infection of pregnant animals (Arricau-Bouvery et al., 2005; Roest et al., 2012). The cause of differences in pregnancy outcome between ruminant species is unknown.

The organism is highly stable in the environment, and due to its resistance to desiccation, infectious organisms may be present for many months after shedding, both in the immediate environment but also on the wool of infected animals (Wattiau et al., 2011). In humans, the primary route of infection is via inhalation of contaminated aerosols, and the majority of human infections are primarily the result of contact with infected livestock. Evidence suggests that close proximity to the source (the goat birthing pen) is the major risk factor associated with infection, and that human activity is largely responsible for the transfer of the infective organisms away from the initial source of infection (Kersh et al., 2013a). However, aerosolised transmission between infected and naïve herds, or the human population and are frequent and often associated with specific environmental conditions, which favour dispersal, including wind, and the topography of the landscape (Mori and Roest, 2018). High wind speeds, open landscapes, high animal densities, and high temperatures were identified as risk factors for transmission of Q fever between Swedish dairy cattle herds (Nusinovici et al., 2017). Likewise, in France, a correlation between human cases of Q fever, high sheep densities and the prevailing Mistral wind has been observed (Tissot-Dupont et al., 2004; Tissot-Dupont et al., 1999). Low consistent wind speed in a flat area of the North Brabant region of the Netherlands was identified as favourable towards the dispersal and transmission of *C. burnetii* to the local population(van Leuken et al., 2015).

While inhalation is the most significant route of *C. burnetii* infection, given the prevalence of *C. burnetii* in milk, dietary ingestion has been studied as a potential route of transmission. Mammals shed *C. burnetii* in milk, and a number of studies have demonstrated the presence of *C. burnetii* DNA in milk and dairy products from cattle (Bauer et al., 2015; Olivas et al., 2016; Pearson et al., 2014; Velasova et al., 2017), goats (Ceglie et al., 2015; Van den Brom et al., 2015a) and sheep (Barkallah et al., 2014). A high rate of *C. burnetii* seropositivity was demonstrated in humans consuming raw goat's milk (Eldin et al., 2013). However, there is an increased likelihood that individuals accessing raw products live in a rural setting, and more likely to be exposed to other potential routes of infection, such as contact with livestock. Despite the widespread shedding of *C. burnetii* in milk, ingestion of dairy products are not seen as a primary route of transmission (Eldin et al., 2013; Gale et al., 2015).

2.3 Human to human transmission

Q fever is thought to be non-communicable, however human-to-human transmission can occur, albeit rarely. As with livestock, birth products from infected women may be a source of infection, to both hospital staff (Raoult and Stein, 1994), and other pregnant women within the same unit (Amit et al., 2014). There is also evidence for sexual transmission (Milazzo et al., 2001) and transmission via blood transfusion is a potential risk. *C. burnetii* is stable in blood samples for at least six weeks at 1-6°C (Kersh et al., 2013b). In a recipient tracing study of blood donors from the Dutch outbreak of 2009, a transfusion recipient in a highly infected area was found to be seropositive after receiving blood from a *C. burnetii* positive donor (van Kraaij et al., 2013). While in this case, the source of infection cannot be categorically determined and the risks of transmission via blood products has been estimated to be low (Oei et al., 2014), infection via this route cannot be ruled out.

2.4 The potential role of arthropods in Q fever transmission

Coxiella burnetii has been identified in over 40 hard bodied and 14 soft bodied species of tick (Eldin et al., 2017) isolated form a wide range of animal host species. Indeed the Nine Mile strain of *C. burnetii* was first isolated in 1935 from the tick *Dermacentor andersoni* in

Montana (Davis et al., 1938). Experimentally, successful tick to animal transmission of *C. burnetii* has been demonstrated in a guinea-pig model of infection (Siroky et al., 2010). However, it is under debate whether ticks play a significant role in the transmission of *C. burnetii,* or whether the presence of the bacteria in the tick population is simply a reflection for *C. burnetii* prevalence in the tick's host species. In the Netherlands' outbreak, ticks were not thought to play a significant role in transmission (Sprong et al., 2012). A recent study of Spanish Ibex suggested that ticks may play a potential role as a vector of infection but modelling suggested a complex interaction between that host, population and environmental factors as drivers of infection in ticks (Varela-Castro et al., 2018).

3. Q fever in Africa

Cases of Q fever have been documented in the African continent for over 60 years. Q fever is an endemic infection across the continent yet we still know relatively little about the prevalence and routes of infection or the health impact of the disease in both livestock and humans. One of the reasons behind this relative paucity of information, and a major barrier towards understanding the impact Q fever is the non-specific nature of symptoms, which are commonly confused for other endemic infections including malaria (Crump, 2014). The was highlighted in a recent 'OneHealth' systematic review (Vanderburg et al., 2014) of Q fever in Africa, which came to the conclusion that '*C. burnetii* presents a real yet underappreciated threat to human and animal health in Africa'.

3.1 Prevalence in Humans

In 1955, the first cases of Q fever were reported in nine countries across the African continent (Kaplan and Bertagna, 1955). Since then a number of studies have demonstrated that the infection is endemic across the continent, but with significant variation in prevalence between countries ranging from 1 % in Chad (Schelling et al., 2003), to 32 % in the Nile Delta in Egypt (Corwin et al., 1993).

Serological evidence of Q fever has been demonstrated in both adults and children; however, rates of *C. burnetii* seropositivity appear higher among young children. High rates of seropositivity in children were observed in Ghana's rural Ashanti Region, where 17 % of two-year-olds were found to be seropositive (Kobbe et al., 2008). In Niger, 10 % of children aged between 1 month old and 5 years of age were seropositive (Julvez et al., 1997). A recent serological survey of 796 children, aged between 1 and 15 years, in Gambia identified *C. burnetii* antibodies in 8.3% (van der Hoek et al., 2013). Intriguingly, they observed that *C. burnetii* seroprevalence was highest among young children (under 4 years), compared with other age groups; however, the reasons for this are still unclear.

Several studies have suggested that Q fever is an important cause of acute fever and pneumonia. *C. burnetii* was identified as the aetiological agent in 5 % of 109 severe pneumonia cases in Tanzania (Rubach et al., 2015). Again in Tanzania, an investigation of febrile patients identified bacterial zoonoses as the underlying cause in 26.2 % of cases, of which 30 % were diagnosed as Q fever (Crump et al., 2013). Within the same patient cohort, malaria was the clinical diagnosis in 60.7% cases, whereas laboratory testing confirmed the diagnosis in only 1.6% of patients. Similarly, Q fever was identified in 8.9 % of paediatric febrile admissions in western Kenya (Maina et al., 2016). Q fever was also identified in 2 % and 9 % of acute hospital admissions across two patient cohort studies of febrile admissions in Tunisia (Kaabia et al., 2006; Omezzine-Letaief et al., 2004), Q fever was diagnosed in 9 % of community-acquired pneumonia cases in Cameroon, demonstrating *C. burnetii* as the second most common etiologic agent of pneumonia, after *Streptococcus pneumoniae* and of equal prevalence as *Mycoplasma pneumonia* (Koulla-Shiro et al., 1997). While the effects of acute infection have been established in several countries, the impact of chronic Q fever in Africa remains largely unexplored. However, in the North African country of Algeria, *C. burnetii* was identified as the cause of 3 % (2/77) of infective endocarditis cases analysed (Benslimani et al., 2005).

3.2 Prevalence in Livestock

Coxiella burnetii is endemic in cattle, sheep, goats, buffaloes and camels across the African continent, though there are significant regional differences, even within countries. In a recent serological survey of 2,699 animals across Egypt, significant inter-species and regional variation was observed (Klemmer et al., 2018). Significantly, camels exhibited the highest rates of seropositivity (40.7%), followed by cattle (19.3%), buffalo (11.2%), sheep (8.9%) and goats (6.8%). Whereas the highest rates of seropositive animals were observed in the Eastern desert (27%), compared to the Nile Delta (16.4%) or Western Desert (17%). Significantly, pasture based production systems also had lower levels of seropositive animals (9.9%) compared with either Nomadic (19.4%) or stationary husbandry. Similarly, nomadic pastoralism was associated with a higher prevalence of *C. burnetii* seropositivity in small ruminants in Baringo County, Kenya (Muema et al., 2017). Camels appear to consistently exhibit high rates of seroprevalence. In Algeria, *C. burnetii* seroprevalence was determined as 71.2% in camels, with age, increased herd size and tick infestation identified as risk factors (Benaissa et al., 2017). Again, despite a lower overall seroprevalence of 18.6%, camel age was determined to be a significant risk factor for *C. burnetii* infection in Laikipia County in Kenya. A further study in Chad also demonstrated 80% seroprevalence in camels, compared to only 4% in cattle, 13% in goats and 11% in sheep (Schelling et al., 2003).

Serology provides evidence of the exposure of livestock to *C. burnetii* but can only provide limited data on the risk of clinical disease. There are however, few published African studies that have directly investigated *C. burnetii* in ruminant abortion. In Niger, 32% of goats with prior history of abortion were found to be seropositive for *C. burnetii* compared with 29% of non-randomly selected goats without abortion (Haumesser and Poutrel, 1973). Likewise, *C. burnetii* positivity was a significant risk factor for cattle abortion in Northern Togo (Dean et al., 2013). In South Africa, *C. burnetii* was observed in aborted lamb foetuses by microscopy (Schutte et al., 1976). *C. burnetii* DNA was identified in the placenta and vaginal swab sample from one aborted goat sample in a study of 109 abortions from Egyptian dairy goats, sheep and cattle (Abdel-Moein and Hamza, 2017). In Tunisia, *C. burnetii* DNA was found in the birth products or vaginal secretions of 19% of small ruminants with a history of abortion (Berri et al., 2009).

3.3 Livestock and human cross infection

A number of studies have associated livestock production as a significant risk factor for the acquisition of Q fever by humans. Direct evidence was provided in a recent study of small ruminants and humans in Gambia, which identified that the presence of a *C. burnetii* positive animal in a compound was a significant risk factor in human infection(Bok et al., 2017). Exposure to cattle, goats, animal slaughter or the consumption of raw milk products were identified risk factors for *C. burnetii* seropositivity in febrile hospital patients in Northern Kenya (Njeru et al., 2016). In Northern Togo *C. burnetii* seropositivity was found to be higher in Fulani pastoralists (45 %) than in non-Fulani (27 %) (Dean et al., 2013), and in Chad, working as a Camel breeder was significantly associated with a positive *C. burnetii* antibody titre (Schelling et al., 2003). In a multi-national study across Africa, higher rates of seropositivity were observed in Mali, Burkina Faso, Nigeria and the Central African Republic, which were the countries under study, exhibited highest densities of ruminants (>100/ humans)

(Dupont et al., 1995). A study of Egyptians in close contact with animals also reported a high overall seroprevalence (16 %) with greater seropositivity among rural (22 %) vs. urban (4 %) residents (Nahed and Khaled, 2012). Similarly, being a rural inhabitant was a significant risk factor for *C. burnetii* seropositivity in an agro pastoral region of Algeria (Lacheheb and Raoult, 2009).

4. The importance of Genotype in understanding the epidemiology of infection *Q fever*

Bacterial genotyping is a key tool in epidemiological investigations, allowing the discrimination of isolates from different sources and thus identify the potential source of infection. Genotyping studies have revealed genetic variability in *C. burnetii* isolates which may exhibit different degrees of pathogenicity or confer host specificity. Genotyping of human and livestock isolates proved effective in identifying the source of human clinical infections during the Netherlands' outbreak (Tilburg et al., 2012a; Tilburg et al., 2012b), which allowed the development of strategies to control the transmission of infection to the human population.

4.1 Genotyping in Europe

Multi locus variable-number tandem repeat (VNTR) analysis (MLVA) has become an established typing technique for a number of bacterial species. Up to 17 different genomic target-regions can be used to discriminate between *C. burnetii* strains, (Arricau-Bouvery et al., 2006; Svraka et al., 2006). MLVA was used to identify a dominant *C. burnetii* genotype circulating within the goat and sheep population during the Netherlands' outbreak, initially using a panel of 3-loci, before a 10-loci MLVA panel was developed (Tilburg et al., 2012a). While a study in human clinical samples used a different set of markers (Tilburg et al., 2012b), alleles in the four overlapping markers were identical, implicating goats and sheep as the possible source of the outbreak. While MLVA analysis initially defined goats as the source of human infection during the Netherlands' outbreak, it was subsequently confirmed by Multispacer sequence typing (MST). The MST method is based upon the analysis of the intergenic regions of genomes. These non-coding regions are not subject to the same selection pressures as the coding regions and are therefore considered to be stable elements of the genome.

An MST typing methodology based upon 10 highly variable spacers was first developed in 2005 (Glazunova et al., 2005) and used to separate 173 isolates into 30 discrete genotypes. A modification of the MST method, real-time PCR protocols to analyse of single nucleotide polymorphisms (SNPs) within the MST loci has also been developed, and used extensively in the USA (Hornstra et al., 2011). MST has shown itself to be a useful tool in both the epidemic situation, but also due to its the widespread use the techniques has allowed direct genotypic comparisons of *C. burnetii* isolates to be made across different host reservoirs throughout the world. In the Netherlands' outbreak, MST33 was identified in both goats and human clinical cases of infection, demonstrating that goats were the probable source of the epidemic (Tilburg et al., 2012a). Further phylogenetic analysis suggested that MST33 genotype may have originated from Germany and entered the Netherlands via France. Conversely, in cattle where *C. burnetii* is endemic, the dominant genotype identified in the Netherlands was MST20. The genotype has been identified extensively in cattle populations, particularly in the USA where the ST20 is the dominant genotype and has been identified in 95.9% of genotyped bulk milk samples, and is not thought to pose a risk to human health (Bauer et al., 2015). While apparently being a cattle adapted genotype (Olivas et al., 2016), and not apparently the source of human disease during the Netherlands' outbreak or in the USA, MST 20 (MLVA I and J) has also been identified in a range of clinical conditions in both humans and animals. These include the presence in human heart valves from France, sheep and goat abortions in the Netherlands (Tilburg et al., 2012a) and UK (Reichel et al., 2012), and cattle abortions in

Hungary (Sulyok et al., 2014b). This apparent discrepancy in pathogenicity and host range between organisms of the same genotype in different geographical locations suggests that despite our current levels of understanding of *C. burnetii* epidemiology, further understanding of local circulating genotypes and/ or greater discriminatory power of genotyping analysis is still required to understand the disease causing potential of the organism in different host species.

The complex nature of circulating *C. burnetii* genotypes within different host species has been examined using a different SNP genotyping protocol, which was developed by a Dutch group during the Netherlands' outbreak (Huijsmans et al., 2011). A total of 10 SNPs were analysed in a total of 14 human and 26 livestock derived samples, using a real-time PCR protocol. This analysis identified 5 distinct genotypes circulating in human and animal populations. The protocol has been used in a number of livestock studies in in Belgium and the USA (Boarbi et al., 2014; Mori et al., 2013; Pearson et al., 2014), the results suggesting a greater degree of variability in circulating *C. burnetii* genotypes in goat populations, but much less variability in the genotypes found in cattle. However the significance of this remains to be elucidated.

4.2 Coxiella burnetii genotypes in Africa

An investigation of the available literature on current *C. burnetii* genotyping techniques (SNP, MST, VNTR, MLVA), via PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and Web of Science (https://wok.mimas.ac.uk/) identified relatively few genotyping studies which have been conducted in Africa (summarised in Figure 1).

Up to 10 distinct MST genotypes have been identified from various hosts (although most are from ticks) and countries. Genotypes MST 2, 6, 16 and 19 have been detected so far in humans

(Hornstra et al., 2011; Mediannikov et al., 2010; Sulyok et al., 2014b). Other hosts include cattle (Rahal et al., 2018), goats (Walter et al., 2014), dogs, rodents (Chitanga et al., 2018) and louse (Louni et al., 2018a; Louni et al., 2018b). While some genotypes (2, 30, and 52) are unique to Africa (Glazunova et al., 2005; Sulyok et al., 2014b), others including MST16 (Sulyok et al., 2014a) and MST20 (Hornstra et al., 2011; Kumsa et al., 2015; Rahal et al., 2018) have been shown to be related to strains from other non-African countries. In addition to the MST data MLVA-typing data is available for two strains from Africa (Morocco and Namibia) showing a distinct pattern with unique regional genotypes (Arricau-Bouvery et al., 2006; Walter et al., 2014).

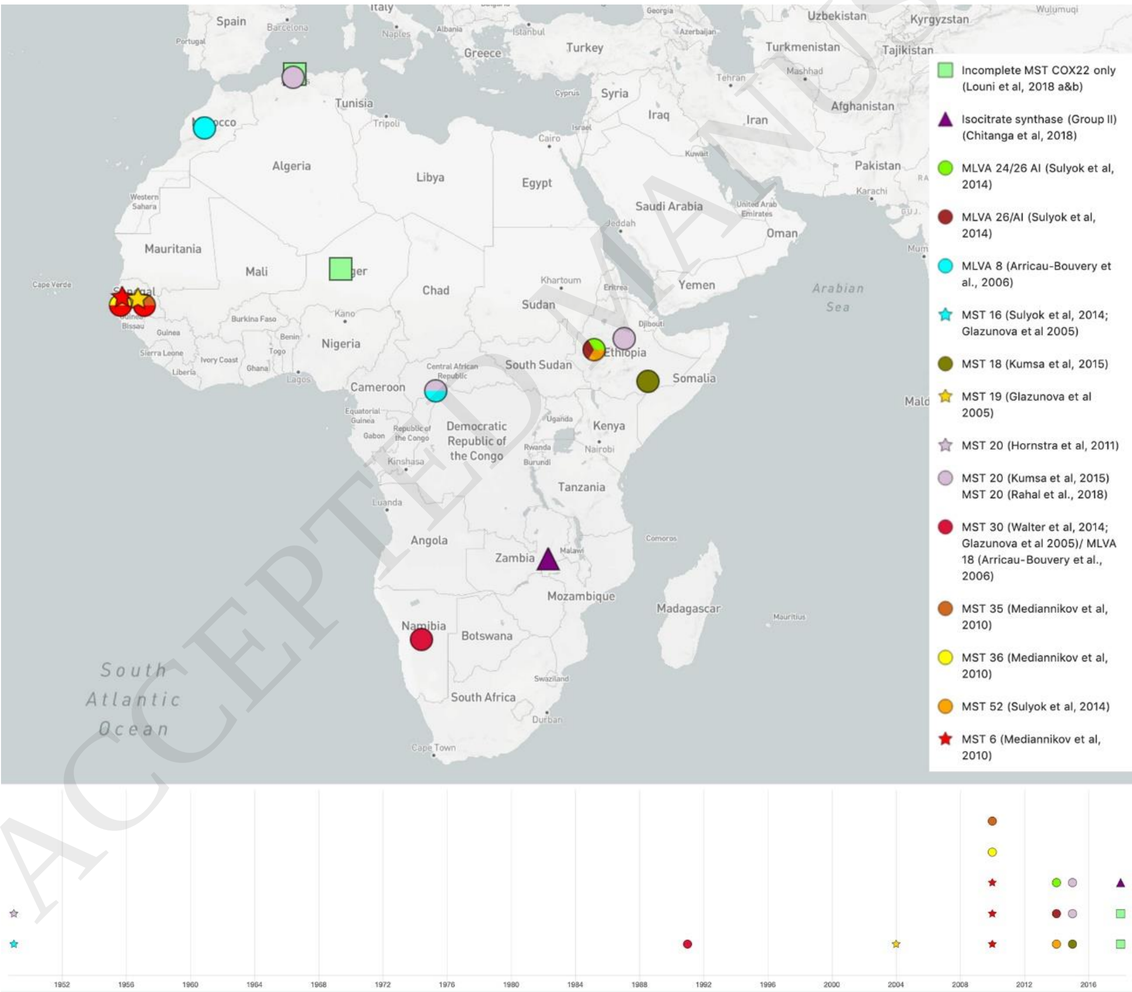

Figure 1: Various *C. burnetii* genotypes identified in Africa. The different colors indicates different genotypes and the shape of the ribbon is indicative of the host; circle (tick), star (human), square (louse), triangle (dogs/rodents). The Namibian isolate is from a goat.

5. Future directions

There is still a relative paucity of knowledge about *C. burnetii* prevalence and the clinical importance of Q fever in both humans and animals across Africa. The majority of studies to date across the African subcontinent have relied solely on serology. While this data is informative in terms of understanding the exposure of populations to the organism, it is of limited use on its own in terms of epidemiological studies, and source tracking information. Much of our current knowledge on Q fever epidemiology is based upon the experience of the Netherlands' outbreak. Here, genotyping proved significant and effective in identifying goats as the definitive source of human infection. This data has been subsequently used to model risk factors and transmission. However, it would be naïve for us to assume that such models of risk, based on Northern European farming practices and climate can be extrapolated across the African continent or even between individual African countries. To date there has been limited genotyping of *C. burnetii* samples across Africa, and studies utilizing genotyping have been skewed towards MST analysis of individual human infections and tick populations (see Figure 1). While livestock infections are largely underrepresented in molecular analysis, the presence of MST20 in Algerian cattle abortions is an important observation (Rahal et al., 2018). However, on its own this is an important observation, but as previously highlighted will only be of limited value unless put into a wider regional context in human and animal health. Given our knowledge of the transmission of *C. burnetii* and differences in the apparent pathogenicity of different genotypes in human and animal populations, the long-term focus must be on integrating serological and molecular genotypic data. Only then will we be able to differentiate between the transmission of *C. burnetii* and the risks of Q fever.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Abdel-Moein, K.A., Hamza, D.A., 2017. The burden of *Coxiella burnetii* among aborted dairy animals in Egypt and its public health implications. Acta tropica 166, 92-95.
2. Amit, S., Shinar, S., Halutz, O., Atiya-Nasagi, Y., Giladi, M., 2014. Suspected person-to-person transmission of Q fever among hospitalized pregnant women. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 58, e146-147.
3. Arricau-Bouvery, N., Hauck, Y., Bejaoui, A., Frangoulidis, D., Bodier, C.C., Souriau, A., Meyer, H., Neubauer, H., Rodolakis, A., Vergnaud, G., 2006. Molecular

characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing. BMC microbiology 6, 38.

4. Arricau-Bouvery, N., Souriau, A., Bodier, C., Dufour, P., Rousset, E., Rodolakis, A., 2005. Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. Vaccine 23, 4392-4402.
5. Barkallah, M., Gharbi, Y., Hassena, A.B., Slima, A.B., Mallek, Z., Gautier, M., Greub, G., Gdoura, R., Fendri, I., 2014. Survey of infectious etiologies of bovine abortion during mid- to late gestation in dairy herds. PloS one 9, e91549.
6. Bauer, A.E., Olivas, S., Cooper, M., Hornstra, H., Keim, P., Pearson, T., Johnson, A.J., 2015. Estimated herd prevalence and sequence types of *Coxiella burnetii* in bulk tank milk samples from commercial dairies in Indiana. BMC veterinary research 11, 186.
7. Benaissa, M.H., Ansel, S., Mohamed-Cherif, A., Benfodil, K., Khelef, D., Youngs, C.R., Kaidi, R., Ait-Oudhia, K., 2017. Seroprevalence and risk factors for *Coxiella burnetii*, the causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria. The Onderstepoort journal of veterinary research 84, e1-e7.
8. Benslimani, A., Fenollar, F., Lepidi, H., Raoult, D., 2005. Bacterial zoonoses and infective endocarditis, Algeria. Emerging infectious diseases 11, 216-224.
9. Berri, M., Rekiki, A., Boumedine, K.S., Rodolakis, A., 2009. Simultaneous differential detection of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. BMC microbiology 9, 130.
10. Berri, M., Souriau, A., Crosby, M., Crochet, D., Lechopier, P., Rodolakis, A., 2001. Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. The Veterinary record 148, 502-505.

11. Boarbi, S., Mori, M., Rousset, E., Sidi-Boumedine, K., Van Esbroeck, M., Fretin, D., 2014. Prevalence and molecular typing of *Coxiella burnetii* in bulk tank milk in Belgian dairy goats, 2009-2013. Veterinary microbiology 170, 117-124.
12. Bok, J., Hogerwerf, L., Germeraad, E.A., Roest, H.I., Faye-Joof, T., Jeng, M., Nwakanma, D., Secka, A., Stegeman, A., Goossens, B., Wegmuller, R., van der Sande, M.A., van der Hoek, W., Secka, O., 2017. *Coxiella burnetii* (Q fever) prevalence in associated populations of humans and small ruminants in The Gambia. Tropical medicine & international health : TM & IH 22, 323-331.
13. Buhariwalla, F., Cann, B., Marrie, T.J., 1996. A dog-related outbreak of Q fever. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 23, 753-755.
14. Ceglie, L., Guerrini, E., Rampazzo, E., Barberio, A., Tilburg, J.J., Hagen, F., Lucchese, L., Zuliani, F., Marangon, S., Natale, A., 2015. Molecular characterization by MLVA of *Coxiella burnetii* strains infecting dairy cows and goats of north-eastern Italy. Microbes and infection 17, 776-781.
15. Chitanga, S., Simulundu, E., Simuunza, M.C., Changula, K., Qiu, Y., Kajihara, M., Nakao, R., Syakalima, M., Takada, A., Mweene, A.S., Mukaratirwa, S., Hang'ombe, B.M., 2018. First molecular detection and genetic characterization of *Coxiella burnetii* in Zambian dogs and rodents. Parasites & vectors 11, 40.
16. Corwin, A., Habib, M., Watts, D., Darwish, M., Olson, J., Botros, B., Hibbs, R., Kleinosky, M., Lee, H.W., Shope, R., et al., 1993. Community-based prevalence profile of arboviral, rickettsial, and Hantaan-like viral antibody in the Nile River Delta of Egypt. The American journal of tropical medicine and hygiene 48, 776-783.

17. Crump, J.A., 2014. Time for a comprehensive approach to the syndrome of fever in the tropics. Transactions of the Royal Society of Tropical Medicine and Hygiene 108, 61-62.
18. Crump, J.A., Morrissey, A.B., Nicholson, W.L., Massung, R.F., Stoddard, R.A., Galloway, R.L., Ooi, E.E., Maro, V.P., Saganda, W., Kinabo, G.D., Muiruri, C., Bartlett, J.A., 2013. Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. PLoS neglected tropical diseases 7, e2324.
19. Davis, G.E., Cox, H.R., Parker, R.R., Dyer, R.E., 1938. A Filter-Passing Infectious Agent Isolated from Ticks. I. Isolation from *Dermacentor andersonii*, reactions with animals, and filtration experiments. Public Health Reports 53, 2259-2282.
20. Davoust, B., Marie, J.L., Pommier de Santi, V., Berenger, J.M., Edouard, S., Raoult, D., 2014. Three-toed sloth as putative reservoir of *Coxiella burnetii*, Cayenne, French Guiana. Emerging infectious diseases 20, 1760-1761.
21. Dean, A.S., Bonfoh, B., Kulo, A.E., Boukaya, G.A., Amidou, M., Hattendorf, J., Pilo, P., Schelling, E., 2013. Epidemiology of brucellosis and q Fever in linked human and animal populations in northern togo. PloS one 8, e71501.
22. Derrick, E.H., 1937. " Q " Fever, a New Fever Entity : Clinical Features, Diagnosis and Laboratory Investigation. Medical Journal of Australia 2, 281-299.
23. Derrick, E.H., 1973. The course of infection with *Coxiella burneti*. The Medical journal of Australia 1, 1051-1057.
24. Dupont, H.T., Brouqui, P., Faugere, B., Raoult, D., 1995. Prevalence of antibodies to *Coxiella burnetti*, *Rickettsia conorii*, and *Rickettsia typhi* in seven African countries. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 21, 1126-1133.

25. Eldin, C., Angelakis, E., Renvoise, A., Raoult, D., 2013. *Coxiella burnetii* DNA, but not viable bacteria, in dairy products in France. The American journal of tropical medicine and hygiene 88, 765-769.
26. Eldin, C., Mahamat, A., Djossou, F., Raoult, D., 2015. Rainfall and sloth births in may, Q fever in July, Cayenne, French Guiana. The American journal of tropical medicine and hygiene 92, 979-981.
27. Eldin, C., Melenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., Mege, J.L., Maurin, M., Raoult, D., 2017. From Q Fever to *Coxiella burnetii* Infection: a Paradigm Change. Clinical microbiology reviews 30, 115-190.
28. Gale, P., Kelly, L., Mearns, R., Duggan, J., Snary, E.L., 2015. Q fever through consumption of unpasteurised milk and milk products - a risk profile and exposure assessment. Journal of applied microbiology 118, 1083-1095.
29. Gikas, A., Kokkini, S., Tsioutis, C., 2010. Q fever: clinical manifestations and treatment. Expert review of anti-infective therapy 8, 529-539.
30. Glazunova, O., Roux, V., Freylikman, O., Sekeyova, Z., Fournous, G., Tyczka, J., Tokarevich, N., Kovacava, E., Marrie, T.J., Raoult, D., 2005. *Coxiella burnetii* genotyping. Emerging infectious diseases 11, 1211-1217.
31. Guatteo, R., Seegers, H., Taurel, A.F., Joly, A., Beaudeau, F., 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. Veterinary microbiology 149, 1-16.
32. Hatchette, T., Campbell, N., Hudson, R., Raoult, D., Marrie, T.J., 2003. Natural history of Q fever in goats. Vector borne and zoonotic diseases (Larchmont, N.Y.) 3, 11-15.
33. Haumesser, J.B., Poutrel, B., 1973. [Rickettsiosis in Niger. Epidemiological survey conducted in the Maradi region]. Revue d'elevage et de medecine veterinaire des pays tropicaux 26, 293-298.

34. Hornstra, H.M., Priestley, R.A., Georgia, S.M., Kachur, S., Birdsell, D.N., Hilsabeck, R., Gates, L.T., Samuel, J.E., Heinzen, R.A., Kersh, G.J., Keim, P., Massung, R.F., Pearson, T., 2011. Rapid typing of *Coxiella burnetii*. PloS one 6, e26201.

35. Huijsmans, C.J., Schellekens, J.J., Wever, P.C., Toman, R., Savelkoul, P.H., Janse, I., Hermans, M.H., 2011. Single-nucleotide-polymorphism genotyping of *Coxiella burnetii* during a Q fever outbreak in The Netherlands. Applied and environmental microbiology 77, 2051-2057.

36. Julvez, J., Michault, A., Kerdelhue, C., 1997. [Serological study of rickettsia infections in Niamey, Niger]. Medecine tropicale : revue du Corps de sante colonial 57, 153-156.

37. Kaabia, N., Rolain, J.M., Khalifa, M., Ben Jazia, E., Bahri, F., Raoult, D., Letaief, A., 2006. Serologic study of rickettsioses among acute febrile patients in central Tunisia. Annals of the New York Academy of Sciences 1078, 176-179.

38. Kampschreur, L.M., Wegdam-Blans, M.C., Thijsen, S.F., Groot, C.A., Schneeberger, P.M., Hollander, A.A., Schijen, J.H., Arents, N.L., Oosterheert, J.J., Wever, P.C., 2010. Acute Q fever related in-hospital mortality in the Netherlands. The Netherlands journal of medicine 68, 408-413.

39. Kaplan, M.M., Bertagna, P., 1955. The geographical distribution of Q fever. Bulletin of the World Health Organization 13, 829-860.

40. Kersh, G.J., Fitzpatrick, K.A., Self, J.S., Priestley, R.A., Kelly, A.J., Lash, R.R., Marsden-Haug, N., Nett, R.J., Bjork, A., Massung, R.F., Anderson, A.D., 2013a. Presence and persistence of *Coxiella burnetii* in the environments of goat farms associated with a Q fever outbreak. Applied and environmental microbiology 79, 1697-1703.

41. Kersh, G.J., Priestley, R., Massung, R.F., 2013b. Stability of*Coxiella burnetii* in stored human blood. Transfusion 53, 1493-1496.

42. Klemmer, J., Njeru, J., Emam, A., El-Sayed, A., Moawad, A.A., Henning, K., Elbeskawy, M.A., Sauter-Louis, C., Straubinger, R.K., Neubauer, H., El-Diasty, M.M., 2018. Q fever in Egypt: Epidemiological survey of *Coxiella burnetii* specific antibodies in cattle, buffaloes, sheep, goats and camels. PloS one 13, e0192188.
43. Kobbe, R., Kramme, S., Kreuels, B., Adjei, S., Kreuzberg, C., Panning, M., Adjei, O., Fleischer, B., May, J., 2008. Q fever in young children, Ghana. Emerging infectious diseases 14, 344-346.
44. Komiya, T., Sadamasu, K., Toriniwa, H., Kato, K., Arashima, Y., Fukushi, H., Hirai, K., Arakawa, Y., 2003. Epidemiological survey on the route of *Coxiella burnetii* infection in an animal hospital. Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy 9, 151-155.
45. Kopecny, L., Bosward, K.L., Shapiro, A., Norris, J.M., 2013. Investigating *Coxiella burnetii* infection in a breeding cattery at the centre of a Q fever outbreak. Journal of feline medicine and surgery 15, 1037-1045.
46. Kosatsky, T., 1984. Household outbreak of Q fever pneumonia related to a parturient cat. Lancet (London, England) 2, 1447-1449.
47. Koulla-Shiro, S., Kuaban, C., Belec, L., 1997. Microbial etiology of acute community-acquired pneumonia in adult hospitalized patients in Yaounde-Cameroon. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 3, 180-186.
48. Kumsa, B., Socolovschi, C., Almeras, L., Raoult, D., Parola, P., 2015. Occurrence and Genotyping of *Coxiella burnetii* in Ixodid Ticks in Oromia, Ethiopia. The American journal of tropical medicine and hygiene 93, 1074-1081.

49. Lacheheb, A., Raoult, D., 2009. Seroprevalence of Q fever in Algeria. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 15 Suppl 2, 167-168.
50. Lang, G., Waltner-Toews, D., Menzies, P., 1991. The seroprevalence of coxiellosis (Q fever) in Ontario sheep flocks. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 55, 139-142.
51. Langley, J.M., Marrie, T.J., Covert, A., Waag, D.M., Williams, J.C., 1988. Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. The New England journal of medicine 319, 354-356.
52. Laughlin, T., Waag, D., Williams, J., Marrie, T., 1991. Q fever: from deer to dog to man. Lancet (London, England) 337, 676-677.
53. Louni, M., Amanzougaghene, N., Mana, N., Fenollar, F., Raoult, D., Bitam, I., Mediannikov, O., 2018a. Detection of bacterial pathogens in clade E head lice collected from Niger's refugees in Algeria. Parasites & vectors 11, 348.
54. Louni, M., Mana, N., Bitam, I., Dahmani, M., Parola, P., Fenollar, F., Raoult, D., Mediannikov, O., 2018b. Body lice of homeless people reveal the presence of several emerging bacterial pathogens in northern Algeria. 12, e0006397.
55. Maina, A.N., Farris, C.M., Odhiambo, A., Jiang, J., Laktabai, J., Armstrong, J., Holland, T., Richards, A.L., O'Meara, W.P., 2016. Q Fever, Scrub Typhus, and Rickettsial Diseases in Children, Kenya, 2011-2012. Emerging infectious diseases 22, 883-886.
56. Malo, J.A., Colbran, C., Young, M., Vasant, B., Jarvinen, K., Viney, K., Lambert, S.B., 2018. An outbreak of Q fever associated with parturient cat exposure at an animal refuge and veterinary clinic in southeast Queensland. Australian and New Zealand journal of public health.

57. Marrie, T.J., MacDonald, A., Durant, H., Yates, L., McCormick, L., 1988. An outbreak of Q fever probably due to contact with a parturient cat. Chest 93, 98-103.
58. Maurin, M., Raoult, D., 1999. Q fever. Clinical microbiology reviews 12, 518-553.
59. Mediannikov, O., Fenollar, F., Socolovschi, C., Diatta, G., Bassene, H., Molez, J.F., Sokhna, C., Trape, J.F., Raoult, D., 2010. *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS neglected tropical diseases 4, e654.
60. Milazzo, A., Hall, R., Storm, P.A., Harris, R.J., Winslow, W., Marmion, B.P., 2001. Sexually transmitted Q fever. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 33, 399-402.
61. Mori, M., Boarbi, S., Michel, P., Bakinahe, R., Rits, K., Wattiau, P., Fretin, D., 2013. In vitro and in vivo infectious potential of *Coxiella burnetii*: a study on Belgian livestock isolates. PloS one 8, e67622.
62. Mori, M., Roest, H.J., 2018. Farming, Q fever and public health: agricultural practices and beyond. Archives of public health = Archives belges de sante publique 76, 2.
63. Morroy, G., Van Der Hoek, W., Nanver, Z.D., Schneeberger, P.M., Bleeker-Rovers, C.P., Van Der Velden, J., Coutinho, R.A., 2016. The health status of a village population, 7 years after a major Q fever outbreak. Epidemiology and infection 144, 1153-1162.
64. Muema, J., Thumbi, S.M., Obonyo, M., Wanyoike, S., Nanyingi, M., Osoro, E., Bitek, A., Karanja, S., 2017. Seroprevalence and Factors Associated with *Coxiella burnetii* Infection in Small Ruminants in Baringo County, Kenya. Zoonoses and public health 64, e31-e43.
65. Nahed, H.G., Khaled, A.A.M., 2012. Seroprevalence of *Coxiella burnetii* antibodies among farm animal and human contacts in Egypt. J Am Sci 8, 619-621.

66. Njeru, J., Henning, K., Pletz, M.W., Heller, R., Forstner, C., Kariuki, S., Fevre, E.M., Neubauer, H., 2016. Febrile patients admitted to remote hospitals in Northeastern Kenya: seroprevalence, risk factors and a clinical prediction tool for Q fever. BMC infectious diseases 16, 244.

67. Nusinovici, S., Hoch, T., Brahim, M.L., Joly, A., Beaudeau, F., 2017. The Effect of Wind on*Coxiella burnetii* Transmission Between Cattle Herds: a Mechanistic Approach. Transboundary and emerging diseases 64, 585-592.

68. Oei, W., Kretzschmar, M.E., Zaaijer, H.L., Coutinho, R., van der Poel, C.L., Janssen, M.P., 2014. Estimating the transfusion transmission risk of Q fever. Transfusion 54, 1705-1711.

69. Olivas, S., Hornstra, H., Priestley, R.A., Kaufman, E., Hepp, C., Sonderegger, D.L., Handady, K., Massung, R.F., Keim, P., Kersh, G.J., Pearson, T., 2016. Massive dispersal of*Coxiella burnetii* among cattle across the United States. Microbial genomics 2, e000068.

70. Omezzine-Letaief, A., Alaoui, F.Z., Bahri, F., Mahdhaoui, A., Boughzela, E., Jemni, L., 2004. [Infectious endocarditis with negative blood cultures]. Archives des maladies du coeur et des vaisseaux 97, 120-124.

71. Pearson, T., Hornstra, H.M., Hilsabeck, R., Gates, L.T., Olivas, S.M., Birdsell, D.M., Hall, C.M., German, S., Cook, J.M., Seymour, M.L., Priestley, R.A., Kondas, A.V., Clark Friedman, C.L., Price, E.P., Schupp, J.M., Liu, C.M., Price, L.B., Massung, R.F., Kersh, G.J., Keim, P., 2014. High prevalence and two dominant host-specific genotypes of*Coxiella burnetii* in U.S. milk. BMC microbiology 14, 41.

72. Pinsky, R.L., Fishbein, D.B., Greene, C.R., Gensheimer, K.F., 1991. An outbreak of cat-associated Q fever in the United States. The Journal of infectious diseases 164, 202-204.

73. Rahal, M., Tahir, D., Eldin, C., Bitam, I., Raoult, D., Parola, P., 2018. Genotyping of *Coxiella burnetii* detected in placental tissues from aborted dairy cattle in the north of Algeria. Comparative immunology, microbiology and infectious diseases 57, 50-54.
74. Raoult, D., Marrie, T., Mege, J., 2005. Natural history and pathophysiology of Q fever. The Lancet. Infectious diseases 5, 219-226.
75. Raoult, D., Stein, A., 1994. Q fever during pregnancy--a risk for women, fetuses, and obstetricians. The New England journal of medicine 330, 371.
76. Reichel, R., Mearns, R., Brunton, L., Jones, R., Horigan, M., Vipond, R., Vincent, G., Evans, S., 2012. Description of a *Coxiella burnetii* abortion outbreak in a dairy goat herd, and associated serology, PCR and genotyping results. Research in veterinary science 93, 1217-1224.
77. Roest, H.J., van Gelderen, B., Dinkla, A., Frangoulidis, D., van Zijderveld, F., Rebel, J., van Keulen, L., 2012. Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. PloS one 7, e48949.
78. Rousset, E., Durand, B., Champion, J.L., Prigent, M., Dufour, P., Forfait, C., Marois, M., Gasnier, T., Duquesne, V., Thiery, R., Aubert, M.F., 2009. Efficiency of a phase 1 vaccine for the reduction of vaginal *Coxiella burnetii* shedding in a clinically affected goat herd. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 15 Suppl 2, 188-189.
79. Rubach, M.P., Maro, V.P., Bartlett, J.A., Crump, J.A., 2015. Etiologies of illness among patients meeting integrated management of adolescent and adult illness district clinician manual criteria for severe infections in northern Tanzania: implications for empiric antimicrobial therapy. The American journal of tropical medicine and hygiene 92, 454-462.

80. Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M., Zinsstag, J., 2003. Brucellosis and Q fever seroprevalences of nomadic pastoralists and their livestock in Chad. Preventive veterinary medicine 61, 279-293.
81. Schneeberger, P.M., Wintenberger, C., van der Hoek, W., Stahl, J.P., 2014. Q fever in the Netherlands - 2007-2010: what we learned from the largest outbreak ever. Medecine et maladies infectieuses 44, 339-353.
82. Schutte, A.P., Kurz, J., Barnard, B.J., Roux, D.J., 1976. Q fever in cattle and sheep in Southern Africa. A preliminary report. The Onderstepoort journal of veterinary research 43, 129-132.
83. Siroky, P., Kubelova, M., Modry, D., Erhart, J., Literak, I., Spitalska, E., Kocianova, E., 2010. Tortoise tick Hyalomma aegyptium as long term carrier of Q fever agent *Coxiella burnetii*--evidence from experimental infection. Parasitology research 107, 1515-1520.
84. Sprong, H., Tijsse-Klasen, E., Langelaar, M., De Bruin, A., Fonville, M., Gassner, F., Takken, W., Van Wieren, S., Nijhof, A., Jongejan, F., Maassen, C.B., Scholte, E.J., Hovius, J.W., Emil Hovius, K., Spitalska, E., Van Duynhoven, Y.T., 2012. Prevalence of *Coxiella burnetii* in ticks after a large outbreak of Q fever. Zoonoses and public health 59, 69-75.
85. Sulyok, K.M., Hornok, S., Abichu, G., Erdelyi, K., Gyuranecz, M., 2014a. Identification of novel *Coxiella burnetii* genotypes from Ethiopian ticks. PloS one 9, e113213.
86. Sulyok, K.M., Kreizinger, Z., Hornstra, H.M., Pearson, T., Szigeti, A., Dan, A., Balla, E., Keim, P.S., Gyuranecz, M., 2014b. Genotyping of *Coxiella burnetii* from domestic ruminants and human in Hungary: indication of various genotypes. BMC veterinary research 10, 107.

87. Svraka, S., Toman, R., Skultety, L., Slaba, K., Homan, W.L., 2006. Establishment of a genotyping scheme for *Coxiella burnetii*. FEMS microbiology letters 254, 268-274.
88. Tilburg, J.J., Roest, H.J., Buffet, S., Nabuurs-Franssen, M.H., Horrevorts, A.M., Raoult, D., Klaassen, C.H., 2012a. Epidemic genotype of *Coxiella burnetii* among goats, sheep, and humans in the Netherlands. Emerging infectious diseases 18, 887-889.
89. Tilburg, J.J., Rossen, J.W., van Hannen, E.J., Melchers, W.J., Hermans, M.H., van de Bovenkamp, J., Roest, H.J., de Bruin, A., Nabuurs-Franssen, M.H., Horrevorts, A.M., Klaassen, C.H., 2012b. Genotypic diversity of *Coxiella burnetii* in the 2007-2010 Q fever outbreak episodes in The Netherlands. Journal of clinical microbiology 50, 1076-1078.
90. Tissot-Dupont, H., Amadei, M.A., Nezri, M., Raoult, D., 2004. Wind in November, Q fever in December. Emerging infectious diseases 10, 1264-1269.
91. Tissot-Dupont, H., Torres, S., Nezri, M., Raoult, D., 1999. Hyperendemic focus of Q fever related to sheep and wind. American journal of epidemiology 150, 67-74.
92. Van den Brom, R., Santman-Berends, I., Luttikholt, S., Moll, L., Van Engelen, E., Vellema, P., 2015a. Bulk tank milk surveillance as a measure to detect *Coxiella burnetii* shedding dairy goat herds in the Netherlands between 2009 and 2014. Journal of dairy science 98, 3814-3825.
93. Van den Brom, R., van Engelen, E., Roest, H.I., van der Hoek, W., Vellema, P., 2015b.*Coxiella burnetii* infections in sheep or goats: an opinionated review. Veterinary microbiology 181, 119-129.
94. van der Hoek, W., Morroy, G., Renders, N.H., Wever, P.C., Hermans, M.H., Leenders, A.C., Schneeberger, P.M., 2012. Epidemic Q fever in humans in the Netherlands. Advances in experimental medicine and biology 984, 329-364.

95. van der Hoek, W., Sarge-Njie, R., Herremans, T., Chisnall, T., Okebe, J., Oriero, E., Versteeg, B., Goossens, B., van der Sande, M., Kampmann, B., Nwakanma, D., 2013. Short communication: prevalence of antibodies against *Coxiella burnetii* (Q fever) in children in The Gambia, West Africa. Tropical medicine & international health : TM & IH 18, 850-853.

96. van Kraaij, M.G., Slot, E., Hogema, B.M., Zaaijer, H.L., 2013. Lookback procedures after postdonation notifications during a Q fever outbreak in the Netherlands. Transfusion 53, 716-721.

97. van Leuken, J.P., van de Kassteele, J., Sauter, F.J., van der Hoek, W., Heederik, D., Havelaar, A.H., Swart, A.N., 2015. Improved correlation of human Q fever incidence to modelled*C. burnetii* concentrations by means of an atmospheric dispersion model. International journal of health geographics 14, 14.

98. van Moll, P., Baumgartner, W., Eskens, U., Hanichen, T., 1993. Immunocytochemical demonstration of *Coxiella burnetii* antigen in the fetal placenta of naturally infected sheep and cattle. Journal of comparative pathology 109, 295-301.

99. Vanderburg, S., Rubach, M.P., Halliday, J.E., Cleaveland, S., Reddy, E.A., Crump, J.A., 2014. Epidemiology of *Coxiella burnetii* infection in Africa: a OneHealth systematic review. PLoS neglected tropical diseases 8, e2787.

100. Varela-Castro, L., Zuddas, C., Ortega, N., Serrano, E., Salinas, J., Castella, J., Castillo-Contreras, R., Carvalho, J., Lavin, S., Mentaberre, G., 2018. On the possible role of ticks in the eco-epidemiology of*Coxiella burnetii* in a Mediterranean ecosystem. PLoS pathogens 9, 687-694.

101. Velasova, M., Damaso, A., Prakashbabu, B.C., Gibbons, J., Wheelhouse, N., Longbottom, D., Van Winden, S., Green, M., Guitian, J., 2017. Herd-level prevalence

of selected endemic infectious diseases of dairy cows in Great Britain. Journal of dairy science 100, 9215-9233.

102. Walter, M.C., Ohrman, C., Myrtennas, K., Sjodin, A., Bystrom, M., Larsson, P., Macellaro, A., Forsman, M., Frangoulidis, D., 2014. Genome sequence of *Coxiella burnetii* strain Namibia. Standards in genomic sciences 9, 22.

103. Wattiau, P., Boldisova, E., Toman, R., Van Esbroeck, M., Quoilin, S., Hammadi, S., Tissot-Dupont, H., Raoult, D., Henkinbrant, J.M., Van Hessche, M., Fretin, D., 2011. Q fever in Woolsorters, Belgium. Emerging infectious diseases 17, 2368-2369.

104. Wegdam-Blans, M.C., Kampschreur, L.M., Delsing, C.E., Bleeker-Rovers, C.P., Sprong, T., van Kasteren, M.E., Notermans, D.W., Renders, N.H., Bijlmer, H.A., Lestrade, P.J., Koopmans, M.P., Nabuurs-Franssen, M.H., Oosterheert, J.J., 2012. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. The Journal of infection 64, 247-259.