FS101016: Q fever risk to human health from the consumption of contaminated unpasteurised milk and milk products.

Milestone Report M02/01: Risk profile: *Coxiella burnetii* in unpasteurised milk and milk products

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Executive summary

Q fever is a zoonotic widespread disease caused by the bacterium *Coxiella burnetii* and is present in cattle, sheep and goats. It is an emerging disease that can cause considerable morbidity and serious long-term complications in humans. Within this risk profile, contextual and background information relevant to the assessment of the risk of human infection with *C. burnetii* from consumption of unpasteurised milk and milk products has been provided. As part of this, other routes of infection have been briefly considered to allow comparison with the milk and milk product route and thus provide an indication of importance of this route in the overall risk of infection with this pathogen.

The risk profile includes research relating to the presence of *C. burnetii* in livestock and milk and milk products, the likely survival of *C. burnetii* during the processing of milk products, the infectivity of *C. burnetii* via the oral route (heavily drawing on research from the 1940/1950s) and also consumption patterns. *C. burnetii* is an obligate intracellular bacterium that relies exclusively on a eukaryotic cell for growth and therefore traditional culture methods cannot be used to detect and enumerate the organism. This results in a number of significant data gaps/deficiencies across the farm-to-consumption exposure pathway and hazard characterisation. However, from this work it can be concluded that the pathogen is endemic in livestock (cattle, goats, sheep) in GB and its DNA is detectable using PCR in raw (and also pasteurised) milk in the UK. It is likely that some of that DNA represents viable pathogen, although the evidence is conflicting. The studies suggest that *C. burnetii* survives well in the environment and information gathered on the methods used to produce raw milk products such as cheese (hard and soft), butter, cream etc. provide no production steps that would result in a significant reduction in the number of *C. burnetii* present, although long maturation at low pHs may give some inactivation in hard cheese. Dose-response data were found to be extremely lacking, although the studies carried out did indicate that, under the conditions given, infection via the oral route is possible but not a frequent occurrence. In addition to this, little information is known for the consumption patterns of raw cattle, goat or sheep milk and milk products. Despite the uncertainties and data gaps/deficiencies there is some strong epidemiological evidence, from the developed world, that cases of Q fever have occurred where consumption of raw milk was the most likely cause. The most recent of these was in Michigan (USA) and involved five individuals. However, suspected milk borne outbreaks have not been reported in GB since the 1950/60s. Although these observational studies are highly suggestive of the consumption of raw milk being the source of the outbreak, there is still uncertainty associated with this link as it has not been proven in any study. With the possible exception of an outbreak in France where raw milk was also consumed, there have been no outbreaks reported due to the consumption of milk products (such as cheese) made from unpasteurised milk, so if cases are occurring they are likely to be sporadic in nature.

Despite the gaps in knowledge, it is concluded that the main route of transmission to humans is not through milk or milk products but via the inhalation of aerosols from the parturient (birth) products of infected animals and/or direct contact with infected livestock; a belief shared by other researchers in this area. This is thought to be attributable to the relatively lower loadings of *C. burnetii* in milk compared to placenta and also the lower infectivity of this pathogen though the oral route compared to the inhalation route.

The overall conclusion of this work is that the risks to humans from consumption of raw milk and milk products are not negligible but they are lower in comparison to transmission via inhalation of aerosols from parturient products and livestock contact. Due to uncertainty in much of the data, a formal risk assessment for infection through milk and milk products cannot be undertaken at present. The gaps in knowledge are compounded by the experimental obstacles in culturing *C. burnetii* associated with intracellular obligatism and therefore data on prevalence of infection between and within herds, survival of the organism and dose-response are lacking. In this respect, a key recommendation of this work is that laboratory methods are developed for detecting and enumerating viable *C. burnetii*.

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1. STATEMENT OF PURPOSE

The objective of this risk profile is to provide contextual and background information relevant to the assessment of the risk of human infection with *Coxiella burnetii* from consumption of unpasteurised milk and milk products. As part of this, other routes of infection will briefly be considered to allow comparison with the milk/milk product route and thus provide an indication of importance of this route in the overall risk of infection with this pathogen.

The approach did not follow a formal systematic review and in that sense literature search criteria are not specified. The approach adopted was to find references and papers in Web of Knowledge and discuss issues with relevant experts.

2. THE ORGANISM

Coxeilla burnetii is an intracellular, Gram-negative pathogenic bacterium which is the causative agent of the disease Q fever. Recent studies have confirmed the ubiquitous worldwide distribution of *C. burnetii* among animal species, within the environment, and even in foods (Hechemy 2012; Massung et al. 2012). Q fever is considered endemic in every country except New Zealand and the continent of Antarctica. The first outbreak of this disease was observed in Queensland, Australia, in 1935 with illness in nine patients who worked in an abattoir in Brisbane (Hechemy 2012).

The main route of infection in humans is inhalation of contaminated aerosols, although oral transmission by contaminated raw milk or dairy products is also possible (EFSA 2010).

C. burnetii is an obligate intracellular bacterium that relies exclusively on a eukaryotic cell for growth. Intracellularly, the organism prospers in an acidified, phagolysosome-like vacuole (PV) and has evolved to replicate in this harsh environment by a mechanism involving acid activation of metabolism (Hechemy 2012; Omsland and Heinzen 2011). However, neutral pH may promote prolonged pathogen viability in the environment while metabolic activation at acidic pH ensures metabolic activity only in a supportive intracellular replication niche. Key characteristics to note are

- *C. burnetii* does not grow outside the intracellular environment of the host cell and relies exclusively on a eukaryotic cell for growth (Omsland and Heinzen 2011).
- Acid activation of metabolism occurs (Hechemy 2012; Omsland and Heinzen 2011)
- Unlike other obligate intracellular bacteria, *C burnetii* has spore-like environmental stability (Minnick and Reghavan 2012).

C burnetii can potentially survive for years in the environment, being highly resistant to chemical and physical stresses, including disinfectants, desiccation, UV light, sonication and osmotic stress (McCaul and Williams, 1981). The organism also shows resistance to heat (Massung et al. 2012) and is certainly more heat resistant than other Gram-negative bacterial pathogens that might occur in raw milk.

The organism has a two stage development cycle, with two distinct morphological variants, or morphotypes namely the large cell variant (LCV) and the small cell variant (SCV). The ability of the organism to survive harsh environmental stresses has been attributed to the resistance of the SCV (Oyston and Davies, 2011).

3. HUMAN DISEASE

3.1 ***Symptoms***

The clinical manifestations of Q fever may be so variable that the disease is often diagnosed only if it has been systematically considered. Acute and chronic disease may be distinguished based on serology. Acute Q fever in humans usually manifests as an asymptomatic or mild flu-like disease with spontaneous recovery (Maurin and Raoult, 1999). However, a small minority of patients present with more serious disease which can lead to serious complications and death. These symptoms include pneumonia, hepatitis, meningo-encephalitis and myocarditis. In some people, the disease can lead to a chronic infection that can manifest years later, even in the absence of primary, acute Q fever symptoms. In these patients, the disease generally manifests as endocarditis (typically in patients with pre-existing valvulopathies), although chronic osteomyelitis, chronic infection of vascular aneurysms or prostheses, lung tumours, pneumonic fibrosis and chronic hepatitis have also been reported (Raoult et al 2000). A chronic fatigue syndrome (CFS) has been reported in 10-20% of patients and severe fatigue levels were present in 52% of patients vs 26% of controls one year after infection in The Netherlands 2007 outbreak (Limonard et al. 2010). However studies in Germany found no increased prevalence of CFS (Strauss et al. 2012). It is generally believed that the incidence of Q fever is under-reported, mainly due to asymptomatic and mild disease and also because the range of symptoms are variable that a degree of clinical suspicion is required for a diagnosis to be made (Maurin and Raoult 1999).

3.2 ***Susceptible groups***

Of the recorded Q fever cases in the UK and in France (Pebody et al 1996, Raoult et al 2000), males of working age (18-65) tend to be more frequently diagnosed, most probably due to occupational exposure on farms or in rural and agricultural settings. However, a serological survey of blood donors in France found that cases with detectable *C. burnetti* antibodies gave a sex ratio of 1:1, compared to a sex ratio of 2.5:1 (male to female) in hospitalised, confirmed cases of Q fever in France from 1982-1990. This suggests that, whilst men and women have an equal risk of infection, men may be more pre-disposed to severe Q fever (Maurin and Raoult

1999). Risk factors for chronic disease include cardiac valve defects and vascular disease, immunocompromised hosts and pregnancy.

3.3 ***Dose response***

Voth and Heinzen (2007) write that the organism is highly infectious with an infectious dose approaching one organism. This is the case through inhalation at least, and possibly through the intraperitoneal route. Indeed the probability of infection through inhalation of a single organism has been estimated to be 90% (Jones et al. 2006).

Transmission by the oral route, however, is controversial (Eldin et al. 2013). A number of human trials have been conducted to determine the link between infected milk and food products with Q fever infection. Krumbiegel and Wisniewski (1970) gave 34 volunteers raw milk that was naturally infected with Q fever over a period of 1 month. The volunteers consumed an average of 4.5 litres of milk under supervision during the month of the trial. The volunteers were followed up for evidence of seroconversion and clinical symptoms. None of the volunteers developed any clinical symptoms even after 12 years. Serum samples were taken 1 month and 2 months after initial ingestion for signs of seroconversion. No detectable antibodies were found in the volunteers by complement fixation test, capillary agglutination test or radioactive precipitin test. The authors concluded that either the milk may have contained a strain that is not infectious to humans or that an inapparent infection without serological response had occurred, which is a phenomenon seen in animals. Another reported trial in Portugal (Fonseca et al, 1949) gave Q fever infected food to 11 patients in an asylum. Of these, two showed signs of seroconversion by complement fixation assay and none developed clinical symptoms. In contrast all 29 persons infected intradermally developed signs of disease. However, only two of 10 volunteers inoculated intranasally were infected, although it is not clear whether those volunteers challenged intranasally received the same doses as those exposed through the oral route.

Langley (1990) reviewed the evidence for transmission of Q fever through breast milk in humans. In humans there is evidence that antibody to *C. burnetii* and the organism itself may be present in breast milk. Indeed studies in India showed that some antibody positive milk samples could infect guinea pigs indicating the presence of viable organism. According to Langley (1990) it is not clear if this may cause disease in the infant.

Studies relating to the oral route have also been conducted in animals and provide additional insight into this route of infection. Babudieri (1959) cites unpublished data on unsuccessful attempts to infect 75 calves, born from serum negative cows, by giving them infected milk. Bell et al. (1949) fed Q-fever infected milk to two calves, aged 3 weeks and 3 months. The 3 month old calf became febrile on the 13th day

and blood samples infected guinea pigs confirming viable pathogen. No symptoms developed in the 2nd calf, although again guinea pigs were infected from the blood. In addition, Langley (1990) cite a 1973 German study (by Schaal and Kleinsorgen) on infection of guinea pigs by the oral route. In this study 8 of 25 animals had antibodies detected by complement fixation. In 5 of the 8 who developed antibodies, rickettsiae[1] were demonstrated in the internal organs. Megaw (1954) summarises a 1953 paper by Combiescu reporting that guinea pigs were readily infected by drinking milk of infected animals.

3.4 ***Knowledge gaps***

A critical knowledge gap is the dose-response for *C. burnetii* in milk or milk products for humans through the oral route. The relevant studies, presented above, do not provide adequate information on the doses given. Therefore it is not known how infectious *C. burnetii* is to humans through the oral route in milk,

3.5 ***Summary and conclusions***

There are very few data on the dose-response relationship for *C. burnetii* via the oral route; the two available studies are old (1949, 1970) and do not provide an indication of the level of dose given. However, the available dose-response studies for infection through the oral route suggest the risks are much lower than through inhalation.

4. THE BURDEN OF DISEASE

4.1 ***Incidence in the UK***

Reports of Q fever are included in the UK Zoonoses Report (Defra, 2012). Table 1 provides a summary of the data. The enhanced surveillance data for England and Wales) were based on diagnosis from HPA reference laboratories, and was seen to increase (across most regions) between 2010 and 2011. This increase is mainly due to better tests being available, allowing processing of more samples with more accurate results, increased awareness in the medical community, together with local outbreaks (Dr J Duggan PHE). The report also provides information on the ratio of males to females (2.5:1 in 2011; 2.1:1 in 2010).

Table 1: Laboratory confirmed reports of Q fever in humans in the UK, 2008-2011

Year	Scotland	Northern	England &	UK total	England &

[1] *Coxiella burnetii* was previously known as *Rickettsia burnetti*

		Ireland	Wales (Enhanced Surveillance)		Wales (Routine surveillance
2008	1	11	56	68	37
2009	2	2	27	31	15
2010	3	0	52	55	23
2011	7	1	104	112	37

*Acute and chronic cases from the Enhanced surveillance database in England and Wales. Data have been updated following a data cleaning project.

**The UK total includes the enhanced surveillance data but not routine surveillance data for England and Wales, as cases reported routinely may also be reported to enhanced surveillance. Enhanced surveillance is the ability of the surveillance team at Health Protection Services, Colindale, to access the Laboratory Management Information System directly rather than waiting to receive the data.

The degree of under-reporting is thought to be largely due to the infection being asymptomatic or causing mild flu-like symptoms. An epidemiological study of Q fever in the UK estimates that only 50 of approximately 700 cases of Q fever pneumonia are reported to the reference centres each year (Pebody et al 1996).

Thomas et al. (1995) reported seroprevalence to *C. burnetii* in five English local authority districts (date not given) as 27.3% in farm workers (105/385) and 10.9% in the general population (43/395) as represented by police and emergency service personnel, suggesting previous exposure. Stanford et al. (1990) report seroprevalence to *C. burnetii* in 28.0% (114 of 406) of randomly selected Northern Ireland farmers (date not given). Most cases in the UK are sporadic (Thomas et al 1995), although a number of outbreaks have occurred including:-

- Solihull in 1989: 147 cases associated with windborne spread from farmland to an urban area (cited by Wilson et al. (2010));
- South Wales in 2002: 95 cases identified in employees of a cardboard factory (van Woerden et al. 2004);
- Scotland in 2006: 110 cases (75 serologically confirmed) in employees at a slaughterhouse and cutting plant (Wilson et al. (2010));
- Cheltenham, England in June 2007: 30 confirmed or probable human cases believed due to windborne spread of *C. burnetii* from nearby sheep farms as the most likely source of infection (Wallensten et al. 2010).

It is interesting to note that intrinsic farm factors, such as production system and management are believed to influence the number of outbreaks in an area (Georglev et al. 2013).

5. SOURCES OF INFECTION

The aerosol route (inhalation of infected fomites) is considered to be the primary mode of human infection with *C. burnetii* (Maurin and Raoult 1999). Connolly et al. (1990) concluded that Q fever was strongly associated with occupation and animal contact on the basis of backgrounds of 443 patients with Q fever in Northern Ireland between 1962 and 1989, although no controls were considered. Thomas et al. (1995) concluded that the risk of Q fever on livestock farms in England is related to contact with the farm environment rather than any specific animal exposure. Infection via *C. burnetii* aerosols may occur directly from direct contact with the excretions and secretions from infected animals. These will include milk, urine, faeces, vaginal mucus, semen and parturient fluids, which may contaminate newborn animals, placenta, or wool (Maurin and Raoult 1999). We now consider these different routes, with particular focus on the milk and milk product related sources.

5.1 ***Milk and milk product related sources***

Although consumption of raw or insufficiently pasteurised milk is very rarely identified as a source of Q fever infection, asymptomatic cattle herds can be considered potential *C. burnetii* reservoirs capable of transmitting the disease to humans (Tilburg et al. 2012). Back in 1992, it was suggested that “Unpasteurised milk and cheese have frequently been found to harbour viable *C. burnetii*, but have rarely been implicated in transmission” (Fishbein and Raoult 1992). Furthermore, Cerf and Condron (2006) challenge the designation of *C. burnetii* as a foodborne pathogen. A recent study in France suggested cheese and yogurt do not pose a public health risk for transmission of Q fever as all cultures from PCR-positive milk products made from unpasteurised milk were negative and mice did not show signs of infection (Eldin et al. 2013). Of potential interest is the recent unpublished finding that the seroprevalence for *C. burnetii* in humans in the Netherlands has decreased from 44.0% in 1983 to 2.4% in 2007, and that changes at dairy industry level did not have an effect (Brandwagt 2012). This is consistent with milk and milk products being a minor pathway for infection with Q fever. Indeed, Maurin and Raoult (1999) in their review of Q fever conclude that although milk may contain large amounts of *C. burnetii*, it is probably a minor route of Q fever acquisition. Maurin and Raoult (1999) go on to write that ingestion (mainly drinking raw milk) is probably a minor factor in the transmission of *C. burnetii* and is now even a point of controversy. However,

there is some epidemiological evidence for transmission by milk. This is now reviewed.

Signs et al. (2012) report on evidence of infection with *C. burnetii* in a small group of regular consumers of raw (unpasteurised) milk from the same dairy in Michigan (USA). Between 9 May 2011 and 22 August 2011, five cases were reported, with serologic testing suggesting acute Q fever. All five regularly consumed raw milk from the same dairy for at least one year. Case 1 had not visited the farm in 9 months, and case 5 had never visited the farm, making raw milk consumption the only common route of exposure in these two cases.

Fishbein and Raoult (1992) reported on an outbreak of Q fever among patients and staff of a psychiatric institution in southern France. Some of the patients and staff left the institution daily to work on a farm where goats were raised for raw milk and cheese production. Seropositive persons were no more likely to have worked on the farm than seronegative ones. Similarly seropositive persons were no more likely to have reported that they consumed unpasteurised dairy products than seronegative ones. However, seropositive persons were significantly more likely ($P = 0.003$) than seronegative persons to have worked on the farm or consumed unpasteurised milk products or both. According to Fishbein and Raoult (1992) some of the seropositive persons had close contact with goats, although the only identifiable exposure of others was the consumption of unpasteurised dairy products. However, the association of seropositivity with exposure to the goats and raw milk products could be confirmed statistically only when these two factors were combined. Furthermore, although the study of Fishbein and Raoult (1992) incriminated unpasteurised dairy products, persons exposed to infected raw milk were less likely to report an associated illness than those who worked with the infected animals.

Brown et al (1968) reported on an outbreak of Q fever in a boys' detention centre in Staffordshire in April 1967. In total, 24 boys and 5 staff were infected. The clinical symptoms included high temperature associated with shivering, sweating, backache, sore throat, headache, chest pains and neck stiffness. One boy displayed symptoms of pneumonia. Serological evidence of Q fever was gained from 19 boys and 5 staff. The centre housed a farm with cattle. Whilst some boys at the centre were involved in agriculture and animal husbandry practices at the farm, none of the infected boys or staff had any contact with the farm. Raw milk from the farm was consumed with cornflakes once or twice a week and the staff took raw milk in their tea. Epidemiological investigations at the farm showed that two out of 20 cows were actively shedding *C. burnetii* and three other cows had antibody titres of >1/10 by CFT in their serum. Neither the farmer, his wife nor two farm hands had antibodies or drank raw milk. In this instance, it was concluded that infection from the raw milk was responsible for the outbreak. Of 443 patients diagnosed with Q fever between 1962 and 1989 in Northern Ireland, 87 (19.6%) drank unpasteurised milk with three drinking goats' milk (Connolly et al. 1990). Unfortunately no statistical analysis was attempted by Connolly et al. (1990) and 46 of the 87 unpasteurised milk drinkers

were also associated with cattle farming in some way. Of the 111 patients who had no animal (cattle, dog, sheep, cat, horse, pig, goat, fowl) contact, 11 drank unpasteurised milk.

Raoult et al (2000) have studied 1,383 confirmed Q fever cases in France from 1985-1998. In 477 cases of acute Q fever, risk factors were analysed and 23% (85/366) of these cases were found to have consumed unpasteurised farm goats' cheese. Of these cases, 16 patients had an isolated fever, 40 had hepatitis, 15 had pneumonia and 11 had hepatitis and pneumonia. 26 patients, who had reported consuming raw milk cheese, were diagnosed with endocarditis. Another epidemiological study of Q fever cases in the UK from 1984-1994 has reported that, out of 1117 cases of Q fever investigated in the study, three were reported to have drunk unpasteurised milk (Pebody et al 1996). Differences in clinical presentation of disease are thought to be due to the route of infection. Hepatitis manifestations are reported more frequently from Ontario, in Andalusia, Spain and in California, US and it has been speculated that this is due to ingestion of contaminated food, especially raw milk (Raoult et al, 2000, Marrie et al, 1996).

Marmion and Harvey (1956) conducted an epidemiological study of two towns in Northern Kent following an outbreak of Q fever in a London Hospital in 1950. Cows' milk purchased by one patient, who resided in one of the towns, was shown to be contaminated with *C. burnetii* and therefore milk was thought to be the cause of the initial outbreak. The epidemiological study of the two towns looked at residents who had reported symptoms of pneumonia or undiagnosed fever between 1948 and 1954. A total of 22 patients from a group of 160 patients identified from medical records had an antibody titre of 1/40 or more to *C. burnetii* by complement fixation (CF) assay. A study of healthy blood donors in the area recruited through the local National Blood Transfusion Service identified two of 240 cases with CF antibody titres of 1/40. Data from the length of residence in the two towns on the risk of infection suggested that Q fever infection was more common amongst new arrivals to the town (<10 years) than in older residents. Of the cases with serological evidence of Q fever infection, one had occupational contact with potential sources of infection, none had residence within 200 yards of a potential source of infection, 5 had visited a potential source of infection (local farm) and 21 (91%) had consumed raw milk. Evidence of Q fever infection was found in the cattle at 2 farms in the nearby vicinity of the towns. From 16 dairies supplying raw and pasteurised milk to the towns, five supplied milk to all those infected with Q fever. Of these, two dairies supplied milk to 13 of the 23 overall cases and were supplied milk from farms known to contain *C. burnetii* infected animals.

Megaw (1954) summarised a 1953 paper by Combiescu that reported an epidemiological study of an outbreak where it was found that infection was usually associated with the drinking of unboiled fresh milk. However, the original paper appears to be in Italian and no details of the country are given.

Schimmer et al. (2011) reported on the risk factors for *Coxiella burnetii* seropositivity in dairy goat farmers' households in the Netherlands in 2009-2010. Interestingly contact with raw milk was a risk factor with odds ratio 2.51 (1.27 – 4.58). However, the meaning of "contact" is not specified and this could reflect contact with fomites containing the milk as ingestion of the raw milk itself is not specified.

The studies above, and especially those described by Brown et al (1968) and Signs et al (2012), give strong evidence of the cause of the outbreaks being the consumption of raw milk, especially for the 2 cases in the Michigan outbreak that had had no recent or no contact with the farm. However, it cannot be definitively proved that infection occurred from consumption of the raw milk. For example, evidence of a dose-response relating risk of infection and quantity of milk ingested is lacking. However despite the uncertainties and data gaps/deficiencies we conclude that there is some strong epidemiological evidence, from the developed world, that cases of Q fever have occurred where consumption of raw milk was the most likely cause.

5.2 ***Milk and milk product production processes***

In this section the processes (with particular relevance to *C. burnetii* survival) in the production of the major milk products made with raw milk in the UK are outlined (with the assistance of the Specialist Cheesemaker's Association). The milk products include::

- Milk
- Butter
- Cheese
- Cream

For each of these products the aim is to identify steps in processing that would act as a barrier, i.e. kill or inactivate the *C. burnetii* bacterium. The processes outlined have been determined from previous FSA research on cheese production (Banks 2006), literature review and discussion with the Specialist Cheesemaker's Association (SCA). Information on how raw milk products are produced is limited, although there was reassurance by the SCA that the processes would be virtually identical regardless of the pasteurisation status of the milk.

Raw milk would typically be stored as for pasteurised milk, except for on-farm production of cheese etc., where the milk may be delivered from parlour to farm without any cooling and used straight away. It is not possible to make yoghurt with raw milk as it requires a period of heating considerably in excess of the milk pasteurisation process in order to denature the protein and provide the correct viscosity. Similarly, clotted cream receives a severe heat treatment.

5.2.1 Raw milk cream

A generic process diagram for the production of raw milk cream is given in Figure 5.1. Apart from the production of clotted cream (which arguably cannot be made with “raw” milk), it appears there are no barriers that significantly reduce levels of viable *C. burnetii* in raw milk cream. There may be some removal of bacterial cells at the point of centrifugation (if cells collect preferentially in the skim milk).

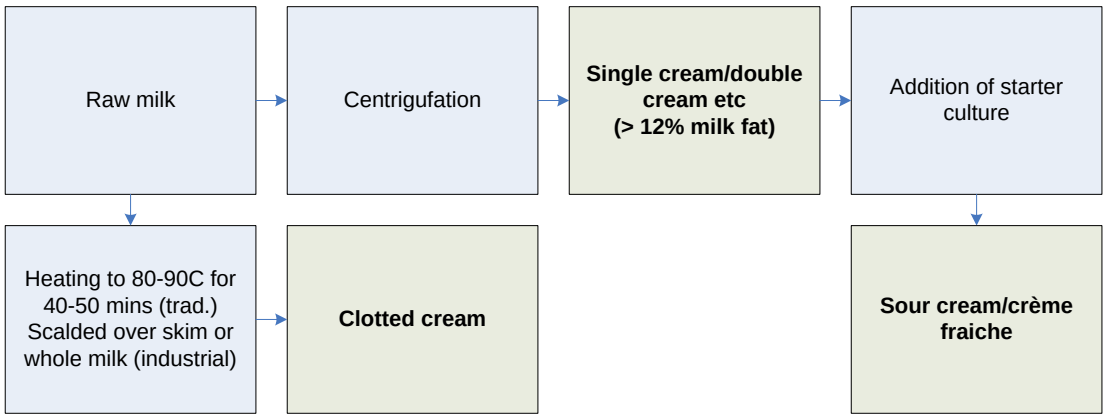

Figure 5.1: Production process for raw milk cream. Blue boxes represent stages of the process, green boxes the products made.

5.2.2 Raw milk butter

A generic process diagram for butter is given in Figure 5.2. As for cream, there is probably no effective barrier for *C. burnetii*, apart from if the bacteria preferentially collect in the buttermilk (in which most of the bacterial population will be drained away with the buttermilk).

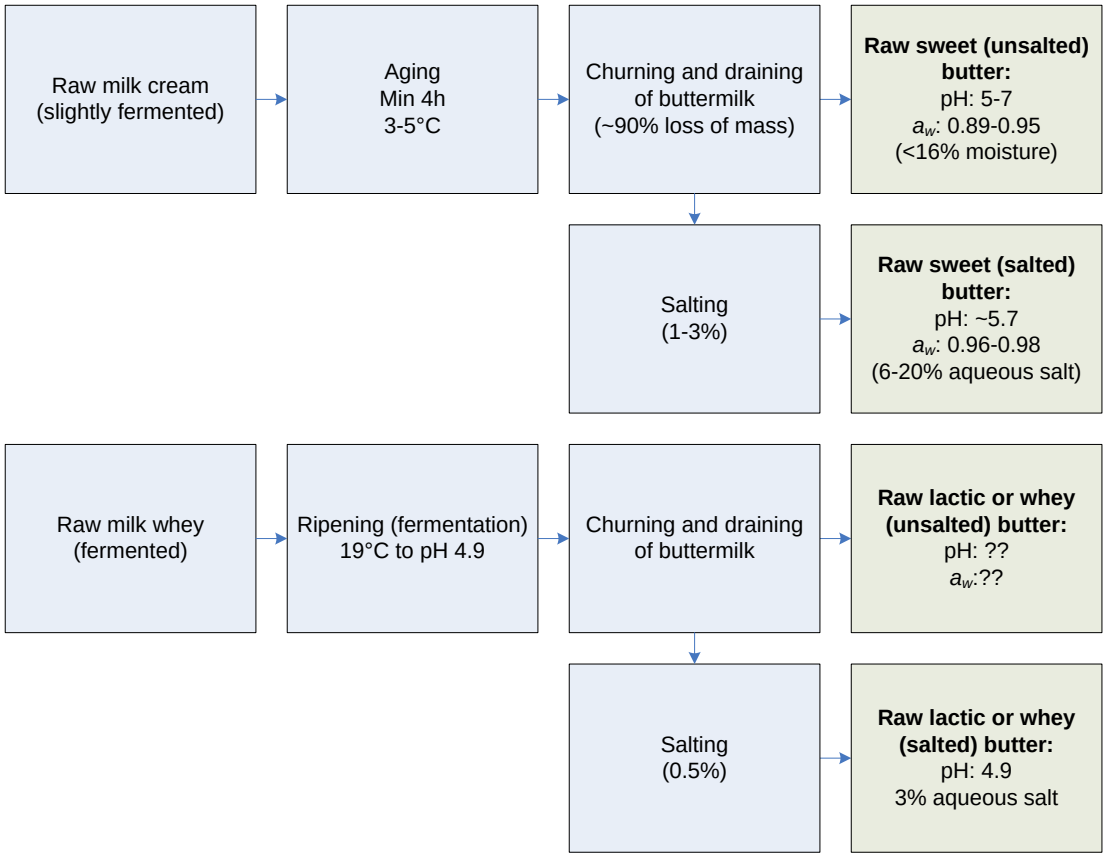

Figure 5.2: Generic process diagram for the main categories of butter. Blue boxes represent stages of the process, green boxes the products made.

5.2.3 Raw milk cheese

A generic process diagram for raw milk cheese is given in Figure 5.3. Again, there is little to suggest that *C. burnetii* would be inactivated by the processes involved in cheese production; only the removal of whey (if bacteria do not preferentially collect in the curds) would be a point where there might be a substantial removal of pathogen from the product. However the time-temperature-pH profiles incurred during the cheese production process and storage may affect the rate of inactivation of the bacteria within the cheese.

As can be seen, there is wide variation in the time-temperature profiles of cheese production, especially between the hard and soft cheeses. We assume raw milk cheese would have similar variability.

Bactofugation may be applied in cheese processing to reduce the levels of bacterial spores and *Clostridium tyrobutyricum,* and has been shown to remove ~90% of total bacteria, 95% of aerobic spores and 98% of anaerobic spores (Giffel and Horst 2004). Microfiltration is more efficient reducing total bacteria and anaerobic/aerobic spores by >99% (Giffel and Horst 2004). However, it is not clear to what extent these processes are applied to raw milk cheeses.

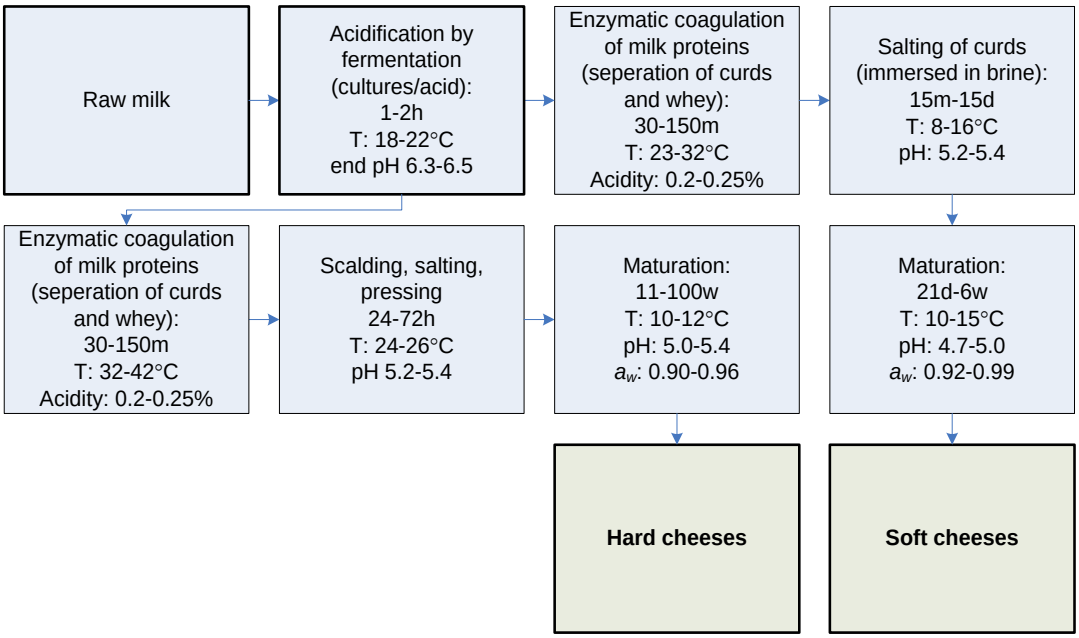

Figure 5.3: Generic process diagram for soft and hard cheeses. Blue boxes represent stages of the process, green boxes the products made.

5.2.4 Production and consumption of raw milk products within the UK

The number of registered producers who provide raw cows' milk for direct human consumption in England and Wales has fallen from around 570 in 1997 to around 100 in 2010 (FSA 2012, para 4.12). The FSA provided data on herd size for 81 dairy cow herds in England and Wales supplying raw milk. The mean number of cows on those farms was 87 with a range of 1 - 300. Similarly, the mean herd size for the 25 goat herds registered as raw milk suppliers was 46, with a range of 2 to 500.

While raw milk for human consumption, distribution and retail may be bottled in dairies in the USA (Loftis et al. 2010; Signs et al. 2012) raw cows' milk for sale in England and Wales is bottled on the farm premises. Thus a distributor may only sell raw cows' milk for human consumption in the containers in which he receives the milk from the farm, with the fastenings of those containers unbroken (Food Hygiene (England) Regs 2006, Schedule 6 para 4 d). The milk can be sold from vehicles used as a shop premises, and also by the farmer at farmers' markets, but no mixing of the cows' milk from different farms occurs.

In a recent survey carried out by the FSA, a total of 62 farms responded that sell raw milk or cream or both in England and Wales; 55 in England and 7 in Wales. Of these 62 farms, 40 sell raw cows' milk totalling 315,339 litres which accounts for 91% of the total raw cows' milk sold (FSA 2013). Of the total raw milk produced and sold (372,174 litres), 65% is sold on the farm and a further 7% through farmers' markets.

Only 5% is sold through distributors. While sales of raw cows' drinking milk are only allowed directly from the farm premises in England, Wales and Northern Ireland (FSA 2012, para 5.7), there are no such restrictions on the sales of raw drinking milk from other livestock species (i.e. goats and sheep).Thus, there are differences in the controls applied to the different livestock species supplying raw drinking milk and cream in England, Wales and Northern Ireland (FSA 2012, para 5.7). However, given the select market for sheep, goat and buffalo milk, it is still likely that the raw milk will be produced and sold as a product from an individual farm (Specialist Cheesemakers' Association, personal communication).

There are no qualitative or quantitative studies on consumption patterns of unpasteurised milk (and the exposure assessment in Appendix 4 draws on data for consumption of pasteurised milk). There are no national data for the amounts of raw milk butter, cream and cheese produced and consumed in the UK. Most production will be on a small scale and produced by hand in a traditional way, so volumes produced/consumed will probably be in the order of a fraction of a per cent of pasteurised butter/cream/cheese production within the UK (Specialist Cheesemakers' Association, personal communication). Previous work by Banks (2006) identified a range of raw milk cheeses, which are listed in Tables 2.2-2.4 in their report.

Limited information on consumption is available from current FSA research. A recent questionnaire surveyed 1,333 consumers, of which 3% currently consume/buy raw milk products. Those buying/consuming raw milk/cream are frequently regular consumers, consuming raw milk/cream on a daily or weekly basis. The majority of raw milk products bought/consumed were produced from cows' milk, but milk from other species, including goats and sheep, was also bought/consumed.

Total quantities of milk and milk products consumed per day are presented in the National Diet and Nutrition Survey (NDNS) from years 1 and 2 (combined) of the rolling programme 2008/9 – 2009/10 (Department of Health 2011).The questionnaire used within the NDNS (NatCen 2013) does not distinguish between pasteurised and raw milk products; hence it is not possible to assess any differences between consumption patterns between the two types. It is also not possible to distinguish between cow, goat and sheep milk products. Assuming typical serving sizes of raw milk products are similar to overall serving sizes according to Department of Health (2011), then we can indicate approximate consumption rates as follows:

- Milk (whole milk, semi-skimmed milk, 1% fat milk, skimmed milk*) – ranging from an average of 297 g/day for 1.5-3 year olds and 127-291 g/day for all other age groups.
- Cream (other milk and cream*) – ranging from an average of 196 g/day for 1.5-3yr olds to 51 – 85 g/day for all other ages.
- Cheese (all cheese) – 12-85 g/day depending on age.

- Ice cream – 9 – 21 g/day depending on age.
- Butter – 1-3 g/day for all ages apart from over 65 yr old (6 g/day).

*categories in parentheses are those quoted in the NDNS survey and include male and female and consumers only, i.e. the lower figures based on both consumers and non-consumers are not presented here.

5.2.5 *Summary of raw milk products processing*

Although there is some evidence for inactivation of *C. burnetii* under relatively high temperatures (i.e. pasteurisation) (see Section 7.4.3) it is unlikely that any of the processes used for raw cheese, cream or butter production would significantly inactivate *C. burnetii*. There are no qualitative or quantitative studies on consumption patterns of unpasteurised milk and milk products in GB. Compared to the production of pasteurised milk/milk products the market for raw milk/milk products is very small. The majority of raw milk is sold by the farmer at the farm gate.

5.3 ***Other sources of infection***

Georglev et al. (2013) conclude that proximity to farm animals and contact with infected animals or their birth products have been identified as the most important risk factors for human disease. Aerosols from the secretions and excretions of ruminants are a major source of contamination for humans (Tissot-Dupont et al. 2004).

In 2007, the Advisory Commission on Dangerous Pathogens (ACDP) considered the risk of occupational exposure to *C. burnetii* in the agriculture and food sector after an outbreak in June/July 2006 at a modern meat processing plant in Scotland involving 93 employees (Wilson et al. 2010). The report concluded that passing through a walkway situated next to the sheep lairage, a nearby store area, and being male were independently associated with being serologically positive for Q fever (Wilson et al. 2010). Passing through other walkways in the boning area, packing area and stores was also associated with more cases than expected.

In cities, the role of slaughterhouses in the spread of Q fever is well known (cited in Brouqui et al. 2004). Indeed Q fever was first recognised as an outbreak of ‘abattoir fever’ in employees of a meatpacking plant in Australia in the 1930s (Wilson et al. 2010) More recently, a slaughterhouse-related outbreak of Q fever in France was related to contaminated waste from the sheep sacrifice for a Christian Easter feast (Brouqui et al. 2004). Brouqui et al. also report an outbreak of Q fever in a homeless shelter in Marseilles, where the main factors exposing persons to *C. burnetii* were an abandoned slaughter-house, used for an annual Muslim sheep feast, and wind.

The resilient nature of *C. burnetii* and consequently its ability to withstand hostile environmental conditions for prolonged periods of time, has led to its incrimination in causing some outbreaks not directly associated with animal contact (Massung et al. 2012), for example, van Woerden et al. (2004) report on an outbreak which was possibly associated with straw. Other authors have also concluded that wind plays a role in *C. burnetii* transmission and two large outbreaks of Q fever have been associated with windborne transmission (Tissot-Dupont et al. 2004). In France, the main peak of Q fever cases occurs in April and May one month after the second lambing season, when the strongest winds blow. During the winter of 1998 to 1999 an increased number of Q fever cases in southern France was associated with an increased frequency of the mistral one month before onset of disease, i.e. shortly after the main lambing season in October and November (Tissot-Dupont et al. 2004).). In a British study (Salmon et al. 1982), Q fever developed in persons who were exposed to contaminated straw, manure and dust introduced by the vehicles that travelled along the road where those persons lived. In a Swiss study (Dupuis et al. 1987), Q fever developed in 415 persons living on a valley road along which sheep were herded to mountain pastures. Wallensten et al. (2010) identified windborne spread of *C. burnetii* from nearby sheep farms as the most likely source of infection in 30 confirmed or probable human cases in Cheltenham, England in June 2007.

Cats and more rarely dogs are a source of infection of humans through placentas and birth products. Marrie et al. (1988) in a case-control study found the strongest association for Q fever in 51 patients was exposure to stillborn kittens. Langley et al. (1988) report an outbreak of Q fever in 1987 among 12 adult members of an urban poker-playing group who were exposed to a parturient cat. There is evidence that *C. burnetii* has been transmitted to humans by exposure to wild rabbits through snaring (Marrie et al. 1986). Thus, 45% of snowshoe hares (n = 22) in the study area had antibodies to *C. burnetii*.

The ubiquitous nature of *C. burnetii* has raised the possibility that transmission might also occur through arthropod-borne routes. The Nobre Marques (NM) strain was isolated from a *Dermacentor andersoni* tick, with virulence of the isolate confirmed through laboratory infection (Massung et al. 2012). To date, no human cases have been directly attributed to tick bites. Similarly, the role of flies has been raised as potentially transmitting *C. burnetii* to livestock rather than directly to humans (cited in Massung et al. 2012).

5.4 ***Knowledge gaps***

There are no reliable production and/or consumption data for raw milk products in GB. However, the scale of production of these products is likely to be several orders of magnitude lower than for pasteurised products. There are no case-control data or

outbreak data which allow quantification of the risks of Q fever infection from drinking raw milk and evidence of a dose-response relating risk of infection and quantity of milk ingested are lacking. In published studies where raw milk is assumed to be attributable to the outbreak of disease, other modes of exposure (e.g. windborne) have not been ruled out. Therefore it has not been definitively proved that infection occurred from consumption of the raw milk. There are few data on infections through consumption of unpasteurised cheese. Indeed, with the possible exception of an outbreak in France (Fishbein and Raoult 1992) where raw milk was also consumed, there have been no outbreaks due to the consumption of milk products such as cheese made from unpasteurised milk, so if cases are occurring they are likely to be sporadic in nature.

5.5 ***Summary and conclusions***

There are a number of routes identified by epidemiological studies for transmission of Q fever to humans. The main routes of transmission are from livestock either through the environment or through direct contact. In this respect aerosolisation and inhalation appear to be important. The resistance of the Q fever organism promotes its transmission through aerosols, and there are suggestions of outbreaks arising from Q fever sources many years after release from an infected animal. The process conditions involved in the manufacture of butter, cream and cheese are unlikely to inactivate the pathogen, although some pathogen may be removed with the whey. In the developed world there is some evidence for human Q fever outbreaks where consumption of raw milk was the most likely cause. There seems to be little data linking Q fever to consumption of unpasteurised cheeses, which suggests the cases are sporadic and, compared to raw milk, are lower.

6. **STUDIES IN ANIMALS**

6.1 ***Prevalence of C. burnetii in livestock in the UK***

6.1.1 *Cattle*

Ryan et al. (2011) reported that 37.9% of bulk milk tank (BMT) samples from cattle herds (n = 290) in the Republic of Ireland were antibody positive for *C. burnetii*. Herds with positive milk samples were randomly distributed in Ireland and no clustering was observed. Paiba et al. (1999) reported that 21% of BTM (n = 373 samples) from dairy herds in England and Wales showed serological evidence of Q fever infection. Valergakis et al. (2012) reported 69.7% of BTM samples from dairy herds in south-west England were positive for *C. burnetii* DNA by PCR. Herd prevalence may not be the same as BTM results because some infected animals

may not shed in milk (Rodolakis et al. 2007) and others may not be lactating. Therefore those data of Paiba et al. (1999) and Valergakis et al (2012) may be used for validation of a risk assessment but not for predicting levels of *C. burnetii* in milk. McCaughey and others (2010) investigated the seroprevalence of Q fever in cattle in Northern Ireland, and found that of 5182 animals tested by ELISA, from 273 herds, 6.2% of animals and 48.4% of herds were seropositive. The percentage of positive herds varied depending on the type of herd and the size of herd. Thus, animals from dairy herds were twice as likely to be seropositive as animals from other herds and small herds had <40% of the odds of seropositivity of large herds (Table 2).

Table 2: Seroprevalence at herd and animal level by herd size for dairy herds in Northern Ireland. Data from McCaughey et al. (2010).

Size of herd	Positive herds/total herds	% positive herds
Large	50/64	78.1
Medium	21/35	60.0
Small	7/22	31.8
All dairy herds	78/121	64.5

Astobiza et al. (2012) studied bulk milk from dairy cattle herds from a Q fever endemic region in northern Spain. Milk from 67% (119 of 178) of herds was positive as determined by the presence of anti-*C burnetii* antibodies. Animals from herds with positive samples had a mean seroprevalence of 8.9% compared to 2.5% in negative herds. The proportion of herds where BTM were positive by PCR was 51.7%. Since milk was analysed by both PCR and ELISA, data from Astobiza et al. (2012) could be used to relate the ELISA prevalence to the presence of DNA.

6.1.2 *Goats*

Van den Brom et al. (2012) report Q fever prevalence in Dutch dairy goat and dairy sheep bulk tank milk (BTM) samples. In 2008, 32.9% of BTM samples tested from farms with more than 200 dairy goats were positive by PCR and 29.8% were positive by ELISA. BTM PCR or ELISA -positive farms had significantly higher within herd seroprevalences than BTM-negative farms. The higher percentage of BTM-positive farms (PCR or ELISA) in the area where the human Q fever outbreak started in the Netherlands in 2007 is consistent with the suspected relation between human cases and infected dairy goat farms.

The seroprevalence in goat herds in Northern Ireland by ELISA was 42.9% (McCaughey et al. 2010).

Lambton et al. (unpublished) tested goat sera collected in 2008 in Great Britain (GB) and reported four goats (n = 522 animals) in four herds (n = 145 herds), tested positive for Q fever by ELISA. Estimates of animal, between-flock and within flock prevalence were 0.887%, 2.97% and 29.9% respectively for goats. There was a tendency for the likelihood of an animal testing positive to increase with goat density.

Between-farm prevalence in goats in the Netherlands was 43.1% with at least one positive goat serum sample on 53 out of the 123 farms. In terms of the number of positive goats (animal level prevalence), seroprevalence was 21.4% (n = 2,828 serum samples from 123 farms) (Schimmer et al 2011). The prevalence of seropositive goats per farm (within farm prevalence) varied between 4.8% and 95.2%.

6.1.3 *Sheep*

McCaughey et al (2010) tested 1,022 sheep from 58 flocks in Northern Ireland and found that 12.3% of animals and 62.1% of flocks were seropositive by ELISA.

Lambton et al. (unpublished) tested sheep sera collected in 2008 in GB and reported 53 sheep (n = 5,791 animals) in 37 flocks (n = 384 flocks) tested positive for Q fever by ELISA. Estimates of animal, between-flock and within flock prevalence were 1.04%, 10.2% and 10.2% respectively for sheep. Likelihood of an animal testing positive for Q fever antibodies increased with total number of sheep in the flock and number of breeding ewes in the flock.

6.2 ***Shedding by infected livestock***

In a naturally infected cattle herd in north-west France, Rodolakis et al. (2007) found 37 of 95 (39%) female cows were shedding *C. burnetii* as detected by PCR testing of vaginal mucus, faeces and milk. There is considerable variation in the probability that a cow is shedding in milk given it is infected. Thus, Rodolakis et al (2007) reported that 92% of the cows that were shedding were positive by PCR on milk samples. However, Guatteo et al. (2012) reported that the numbers of cows excreting in milk at days 14, 21 and 28 after abortion were 9/24, 6/24 and 7/24, respectively (using a cut-off of Ct value >40 to indicate a negative by PCR). Furthermore shedding is variable and Guatteo et al. (2012) identified some cows as persistent shedders in that they were shedding relatively high levels on all three occasions. While Guatteo et al. (2012) only looked at a two week interval, Enright et al. (1957) showed that shedding in cattle can occur for 205 – 405 days. Rodolakis et al. (2007) compared the shedding of *C. burnetii* in milk of dairy bovine, caprine and ovine herds. Shedding

of *C. burnetii* differs among ruminant species, milk being the primary route in cattle and goats (Rodolakis et al. 2007). Sheep shed mainly in the faeces and vaginal mucus and to a lesser extent in milk. The shedding in milk was sometimes intermittent and several animals shed the bacteria but were negative by ELISA. Guatteo et al. (2012) reported concomitant shedding via both milk and vaginal mucus in 46% of detected shedder cows 14 and 28 days after abortion. In the study of Guatteo et al. (2012) 21% of the cows were persistent shedders (defined as shedding on all three occasions) regardless of shedding route from day of abortion to day 28.

Rousset et al. (2009) reported that *C. burnetii* was shed into vaginal mucus, faeces and milk of 44%, 21% and 38%, respectively, of goats that aborted and 27%, 20% and 31%, respectively of goats that delivered normally in infected herds, although the differences between the two groups were not statistically significant. Rousset et al. (2009) also reported two opposite shedding trends for milk and vaginal/faecal routes with respect to serological findings. Thus unlike positive milk shedding, positive vaginal and faecal shedding were observed more often in animals that were weakly positive or negative by ELISA or indirect immunofluorescence assay.

Modelling of Q fever spread within dairy cattle herds has shown that antibody status seems to be a key point in bacterial spread; thus antibody positive cows shed for longer than antibody negative cows (Courcoul et al. 2010).

6.3 ***Tissues targeted by C. burnetii***

Monocytes and macrophages are the major targets of *C. burnetii* (Amara et al. 2012) and spread around much of the body. Understanding the tissues where *C. burnetii* is housed and survives may give a better understanding of the relative loadings in placenta, milk, faeces and vaginal mucus in livestock, although extrapolation of findings from one species to another may not be appropriate, particularly since shedding patterns in faeces, milk and vaginal mucus differ for different livestock species (Rodolakis et al. 2007). According to Amara et al. (2012) three tissues house *C. burnetii*. These tissues are:-

1) The bone marrow;
2) Adipose tissue; and
3) Placenta.

Trophoblasts are found in the placenta. Amara et al. (2010; 2012) have observed that *C. burnetii* infects and persists within a trophoblastic cell line, although the site of *C. burnetii* within the placenta remains unknown. The placenta is a tissue rich in macrophages and Amara et al (2012) recently found that placental macrophages habour *C. burnetii* (Amara et al. 2012).Huge numbers of *C. burnetii* can be released

into the environment via livestock birth products (Tilburg et al. 2012; Babudieri 1953; 1959). Indeed one gram of ovine placenta contained >10^9 hamster or guinea pig ID_{50}s (Welsh et al. 1951).

For the purpose of this risk profile it is relevant to consider how *C. burnetii* gets into milk.In cattle, *C. burnetii* colonises mammary glands and can be excreted in milk for months after infection (see Loftis et al. 2010). However, while *C. burnetii* may target adipose tissue in the mammary gland, there is no evidence that *C. burnetii* infects the glandular tissue *per se* and the mammary epithelial cells which secrete proteins into the milk ducts. Within the tissues, the monocytes and macrophages are the major targets of *C. burnetii* (Amara et al. 2012) and these cells would need to get through the epithelial layer in the mammary gland ducts and so enter the milk. Certainly immune cells including macrophages occur in bovine milk (Paape et al 2003). However, it is unlikely that *C. burnetii*-infected monocytes and macrophages are specifically recruited to milk by a specific tissue tropism over non-infected macrophages. So the occurrence of *C. burnetii*-infected macrophages in milk would tend to be random rather than active selection. This would again be consistent with low levels of *C. burnetii* being present in milk compared to the placenta for example.

6.4 ***Knowledge gaps***

There are some good data on the presence of *C. burnetii* in livestock for Northern Ireland and for *C. burnetii* in cattle in England and Wales. However, there are fewer published data for sheep and goats in England and Wales. For cattle, goats and sheep there are some data on the proportions of infected animals which shed in milk. The main knowledge gaps are in the variation in levels shed by individual animals over time and the length of shedding. There is little knowledge on how the organism is distributed within the tissues of the infected animal and in particular the mechanisms for how the pathogen gets into milk itself. The gaps in knowledge on prevalence are compounded by the experimental obstacles in culturing *C. burnetii* associated with intracellular obligatism (Omsland and Heinsen 2011). The advent of PCR has enabled detection of *C. burnetii* DNA and even quantification of *C. burnetii* DNA in BTM as used by Valergakis et al (2012) for dairy cattle in GB. This gives an indication of prevalence. However, the problem with PCR is that it gives no information on viability and mouse bioassay or non-routine culture techniques have to be used to confirm viability of positive milk samples (Loftis et al. 2010). ELISA techniques as used by Lambton et al. (unpublished) and Paiba et al. (1999) may over-estimate prevalence because animals may be sero-positive for life, although some may later convert from sero-positive to sero-negative.

6.5 ***Summary and conclusions***

C. burnetii is endemic in GB dairy cattle herds which, in the case of herds in Northern Ireland at least, have higher prevalences than beef cattle herds. The DNA of the pathogen, as detected by PCR, appears ubiquitous in BTM. The main tissue source of *C. burnetii* is the placenta where levels can reach 10^9 per gram of tissue. Cows and goats excrete the bacteria mainly in milk while ewes shed mostly in faeces and vaginal mucus. Shedding occurs in milk in infected cattle for long periods and persistent shedders have been identified.

7. STUDIES IN MILK AND MILK PRODUCTS

7.1 ***Presence of C. burnetti in commercially available raw milk***

Loftis et al. (2010) have confirmed the viability of *C. burnetii* in at least two and maybe four of six PCR-positive commercial, raw milk samples. For raw milks in the USA, Loftis et al. (2010) found that 42.9% of purchased raw milk samples were PCR positive for *C. burnetii*, and 2 of the 6 samples infected live mice confirming the pathogenic potential. Specifically Loftis et al. (2010) purchased 21 unpasteurised milk samples from 20 different dairies in 12 US states. These included 7 goat milk dairies and 13 cattle milk dairies. Of the 21 raw milk samples, 9 (42.9%) contained DNA from *C. burnetii*, including 2 samples of cow milk purchased from the same dairy about one month apart. One of 7 samples of goat milk (14.3%) and 8 of 14 (57.1%) samples of cow milk tested positive. Isolation of the pathogen in mice (by bioassay) was attempted for six positive samples. Two of four mice inoculated with one sample and four of five mice inoculated with another sample became infected with *C. burnetii* which was successfully passed onto naïve mice. Two other milk samples may have caused infection in live mice as small amounts of *C. burnetii* DNA were found in the spleen. Other bacterial infections may have prevented isolation. These results confirm the viability of *C. burnetii* in commercially sold raw milk.

Tilburg et al. (2012) report that real time PCR shows the widespread presence of *C burnetii* DNA in a broad range of commercially available milk and milk products. Indeed Tilburg et al. (2012) reported that 88 of 116 (76%) of commercially available milk samples (typically semi-skimmed milk from cows obtained from large supermarket chains) contained significant amounts of *C. burnetii* DNA. The study sampled milks for 28 countries including the UK for which one sample of semi-skimmed milk was PCR-positive. The study also found *C. burnetii* DNA in coffee creamers and milk powder.

7.2 ***Presence of C. burnetii in milk products***

To date, three papers have been published reporting on prevalence of *C. burnetii* DNA in cheeses by PCR. In Italy Capuano et al. (2012) reported 31.2% of hard cheese samples (n = 77) to be PCR-positive compared to 13% of soft cheeses (n = 92). This, however, could include non-viable bacteria and Capuano et al. (2012) note that their study is limited in not confirming the PCR results in terms of the viability of *C. burnetii* from the tested samples. Indeed, the viability of *C. burnetii* appears to be lost in cheese samples. ThusHirai et al. (2012) and Eldin et al. (2013) reported no viable *C. burnetii* in 7 and 9, respectively, unpasteurised milk cheeses which were PCR-positive. Capuano et al. (2012) also reported 39% of cheese from cows' milk to be PCR positive compared to 26.8% sheep and goat cheese. Hirai et al. (2012) describes a method for detection of *C. burnetii* in cheese with a detection limit of 600 bacteria per gram. Using this method 28 of 147 cheese samples from commercial markets in Japan were positive. However, samples from the seven unpasteurised milk cheeses which were PCR-positive were non-viable in mice. Eldin et al. (2013) collected 178 cheeses (from supermarkets, dairies and artisanal producers) in France and found 117 (65%) were positive by PCR. Of these 117 cheeses 39 were unpasteurised. Of these, nine samples which were strongly positive by PCR gave negative results by cell culture and five were tested further for viability by inoculation in mice did not result in infection of the mice.

The advent of PCR has had a clear disadvantage on obtaining data useful for risk assessment with regard to the viability. In this respect, the guinea pig infection assays undertaken before the advent of PCR offer much greater value for risk assessment.

7.3 ***Measured levels of C. burnetii in milk and milk products***

7.3.1 *Data for cows' milk*

Brandwagt (2012) writes that the number of *C. burnetii* in unprocessed milk is unknown. To date and through an extensive review of the literature, two key papers (Bell et al. 1949; Enright et al. 1957) have been found giving counts of viable *C. burnetii* densities in milk from cattle.

Bell et al. (1949) attempted infection of nine lactating cows by different routes. Four cows were inoculated with Q fever infected yolk sac cultures through the teat canal of the two left quarters of the udder. Samples of milk were tested for infectivity in guinea pigs. *C. burnetii* was isolated from the blood of all four test cows on two or more of the first 5 days, but not from blood taken after this time – showing that infection had taken place. Repeated titration in guinea pigs of milk from a single quarter of the cow sacrificed on the 63rd day revealed the presence of *C. burnetii* in dilutions of at least 10^{-5} and 10^{-4} on the 2nd and 7th days post-inoculation,

respectively, and thereafter at 5 day intervals at a dilution of only 10^{-1}, i.e. higher dilutions were negative. Unfortunately the volume of milk injected is not stated by Bell et al. (1949).

Bell et al. (1949) thus reported a maximum of 10^5 guinea pig infective doses /ml in milk. However, it should be noted that following the inoculation with *C. burnetii* an acute mastitis accompanied by a marked systemic reaction was produced in that one cow which was slaughtered on day 63. The mastitis was accompanied by a marked increase in the cellular constituents of the milk. The cell count of the milk rose to a peak of 20 to 30 million per ml and consisted chiefly of polymorphonuclear leukocytes (PMNs). It should be noted also that the concentration of butter fat increased in the milk and more importantly that the total daily milk production decreased markedly. Thus the mastitis may have caused the decrease in milk volume and hence the apparent increase in densities of *C. burnetii* measured in the milk. Thus the 10^5/ml of milk reported on day 2 by Bell et al. (1949) may be an overestimate caused by low milk volume due to mastitis. Furthermore, mastitis causes infiltration of macrophages (the cells in which *C. burnetii* replicate during infection) into milk (Paape et al 2003). Indeed the role of the macrophages is to mop up the PMNs before their chemicals damage the delicate lining of the mammary gland which would reduce the number of milk secretory cells (Paape et al. 2003). Thus the mastitis may increase the measured densities of *C. burnetii* by two mechanisms i) increasing the number of *C. burnetii*-infected macrophages actually in the milk and ii) decreasing the volume of milk produced. From a risk assessment perspective, the milk from cows with mastitis would not be used in the food chain.

Enright et al. (1957) provide quantitative data on levels of viable *C. burnetii* in the milk of individual cows from a naturally infected herd. Overall 18 of 137 cows in the herd were infected. To quantify viable *C. burnetii*, Enright et al. (1957) used guinea pig bioassay, which unlike PCR, gives information on the viability of the organism in milk. Thus, while PCR can give quantitative data on the number of DNA copies, it gives no information on the viability of the organisms. Thus, Loftis et al. (2010) had to test viability of PCR-positive raw milk samples by their ability to infect mice. Furthermore, Enright et al. (1957) went on to titrate levels of infectivity in guinea pigs thus quantifying the levels of infectivity. Enright et al. (1957) define an infective guinea pig dose as the minimum number of *C. burnetii* required to infect a guinea pig by intraperitoneal inoculation. Two consecutive passages were done in guinea pigs to allow for antibody generation from dead bacteria in the first assay and the results do indeed represent viable bacteria. The results obtained by Enright et al. (1957) for individual cows are summarised inTable 3.

Table 3: Summary of data for number of *C. burnetii* infective doses in milk of individual cows from a naturally infected herd. Data from Enright et al. (1957)

Study	Sample Information	Guinea pig intraperitoneal infective dose 50%s
18 positive cows from a naturally infected herd (n = 137) i.e. 86.9% negative	3 cows	1,000 per 2 ml
	5 cows	100 per 2 ml
	5 cows	10 per 2ml
	5 cows	1 per 2 ml

In addition, Enright et al. (1957) measured *C. burnetii* infectivity (in terms of guinea pig infective dose 50%s) in 109 raw milk samples obtained from a creamery upon delivery from the dairy farm. Unlike the individual cow samples in Table 3, these were composite samples of the milk from one farm. Of these, 8 samples contained viable *C. burnetii*. In addition, 376 retail milk or cream samples were taken from a different creamery; each sample being from a composite from different producers supplying the creamery. Of the 376 retail samples, 14 raw and one pasteurised sample contained *C. burnetii*. In total 22 of 485 samples (4.5%) were positive with 95.5% being negative. The maximum number of organisms demonstrated in those samples was 1,000 guinea pig infective doses per 2 ml.

Enright et al. (1957) considered the maximum amount of infectivity in cows' milk and reported that the milk of an experimentally infected dairy cow contained 10,000 infective guinea pig doses of *C. burnetii* (presumably this is per 2 ml). The animal had been infected by introducing the Henzerling strain of the organism into the teat canal. The positive milk sample was obtained on the ninth day after inoculation. According to Enright et al. (1957), information from other investigators (not cited) revealed that 10,000 infective guinea pig doses per ml of milk was the maximum. Enright et al. (1957), however, considered 100,000 infective guinea pig doses per 2 ml of milk as an appropriate number to use in their pasteurisation study. First, not all breeds of dairy cows were investigated and some might be capable of shedding greater numbers in their milk than those tested. Second, the maximum recorded in the composite milk samples (discussed above) was 1,000 guinea pig infective doses per 2 ml, although 95.5% contained 0 organisms suggesting some animals were shedding greater numbers of *C. burnetii* in their milk than that number contained in

1,000 infective guinea pig doses. This is consistent with 86.9% of individual cows in a herd being negative.

As well as the data from Bell (1949) and Enright et al. (1957), a more recent study by Lopez-Gatius et al. (2011) describes the findings of PCR results for *C. burnetii* in the BTM with an excretion level higher than 10^4 Coxiella/ml. The PCR method has been used to estimate titres in cows' milk by Guatteo et al (2007). Titres were determined by comparison of PCR results with those from solutions with a known *C. burnetii* concentration obtained by serial dilution of an external positive control. Guatteo et al. (2007) give titre estimates for milk and mucus in shedding cows. Titres vary according to the shedding pattern (e.g. persistent shedding, intermittent shedding) with median values of 3.34 $\log_{10}$/ml of milk for persistent shedding cattle and a maximum of 5.33 $\log_{10}$/ml of milk (n = 98 cows). These loadings were higher than for intermittent shedders with median and maximum values of 1.38 and 2.64 $\log_{10}$/ml of milk respectively (n = 28). The viability of the titres is not known. It should be noted that the target for PCR is often a sequence which is present in multiple copies in the bacterial genome, thus complicating the enumeration of organisms from the number of DNA copies detected by PCR. Furthermore the number of copies of target sequence may vary from strain to strain of pathogen.

In addition, Valergakis et al. (2012) present *C. burnetii* DNA levels/ml from PCR data for cattle BTM samples taken in south-west England. Of the 155 BTM samples, 3.9 per cent, 10.3%, 18.1%, 24.5% and 12.9% had *C burnetii* DNA levels of 2.0–2.5, 2.5–3.0, 3.0–3.5, 3.5–4.0 and >4.0 log10 units, respectively. As above, unfortunately, the viability of the titres is not known.

7.3.2 Data for goats' milk

Roest et al. (2012) give semi-quantitative PCR data on excretion of *C. burnetii* in goats' milk. Sampling was started at parturition and DNA was detected in the milk until 38 days post parturition. The results of Roest et al. (2012) are interesting in confirming that DNA levels are highest in the vaginal mucus, placenta and uterus of infected goats compared to a range of other tissues in the goat. Furthermore, the levels of *C. burnetii* DNA in milk are lower than in faeces and vaginal mucus. The positive milk sample was taken 9 days post partum (dpp) with PCR negatives in milk being recorded after that up to 95 dpp. The semi-quantitative PCR data of Roest et al. (2012) thus confirm that *C. burnetii* loadings are highest in placenta tissue and vaginal mucus and very low in milk in goats.

7.3.3 Data for cheese and other milk products

To date, no published information has been found giving counts of viable *C. burnetii* in cheese or any other milk products. Studies in the 1950s investigated survival of

viable *C. burnetii* in butter but the results were presence/absence and not quantitative (Zubkova 1957). Similarly studies of survival of viable *C. burnetii* in cottage cheese (Sipka 1958) were not quantitative.

Eldin et al. (2913) reported a mean of $10^{3.9}$ DNA copies/ml and $10^{3.6}$ DNA copies/ml for unpasteurised and pasteurised products (mostly cheese) respectively. Indeed, significantly more unpasteurised samples contained more than 1,000 DNA copies/ml. Fourteen products (including 9 unpasteurised) containing more than 10,000 DNA copies/ml showed negative results in culture. 9 of the strongly positive samples (5 unpasteurised) were inoculated into mice and all animals showed no signs of illness for infection from their spleens. This demonstrates that although there can be a large amount of DNA present in cheese, this DNA may not represent viable pathogen.

7.4 ***Survival in milk and milk products***

7.4.1 *Survival time*

There are limited data on *Coxiella* survival under various conditions and what data is available is quite old (Robert Heinzen, pers comm 24/1/13). The table on resistance taken from Williams (1991) (see Table 4) is about the extent of current knowledge (Robert Heinzen, pers comm 24/1/13). Robert Heinzen's recommendation was, given the lack of knowledge, that AHVLA/FSA conduct further experiments. RIVM in the Netherlands have no information on survival of *Coxiella* in milk or cheese (Wim van der Hoek, pers comms. 28 Jan 2013).

Data from Hirai et al. (2012) using mice studies suggest that viability of *C. burnetii* was lost in PCR-positive unpasteurised cheese samples. However, *C. burnetii* may survive in other cheese samples. Thus, Sipka (1958) used cows' milk infected with *C. burnetii* which in dilutions of 1:100 was still pathogenic for guinea pigs, for the preparation of a cottage-type cheese, which retained its infectivity for guinea pigs for a period of observation of 42 days.

Jellison et al. (1948) reported the presence and persistence of infectious *C. burnetii* in butter made from naturally infected cows' milk. The method was seroconversion of guinea pigs injected intraperitoneally with the butter or milk. Refrigerated butter stored at below freezing temperature was still infectious to guinea pigs 41 days after preparation. Sera from four guinea pigs injected with freshly churned butter made from naturally infected raw milk were positive at high titre.

C. burnetii survived in milk (dried 37°C) for 30 – 60 days and in cheese made from infected milk for 17 – 46 days (Babudieri and Moscovici 1950). *C. burnetii* survived in sterile milk at room temperature for 125 days (Zubkova 1957). Babudieri (1953) notes that that *Coxiella* in cheese is no longer a source of infection after 46 days presumably referring back to the Babudieri and Moscovici (1950) study. According to

Brouqui et al. (2004) *C. burnetii* has been shown to survive for >40 months in skim milk at room temperature, citing the review by Maurin and Raoult (1999). However, Maurin and Raoult (1999) do not give any such data or even mention this.

Table 4: Resistance of *C. burnetii* (from Williams, 1991)

TABLE 2
Resistance of *Coxiella burnetii* Under Various Conditions

Year	Experimental conditions	Temperature	Survival period or result	Ref.
1938	In leptospira media (cell-free)	28°C	109 d or >	38
1940	"Lyophilized" guinea pig spleen	—	5 months or >	39
1943	In the tick, *Ornithodorus hermsi*	—	979 d	40
1948	Tick feces	Room temp.	586 d or >	41
1949	Dried guinea pig blood	Room temp.	182 d or >	42
	Dried guinea pig urine	Room temp.	49 d	
1948	Butter made from unpasteurized cream	—	41 d	43
1949	Infected milk: 30 min at	71.5°C	Killed	44
	15 min at	61.5°C	Some survive	
1952	Infected milk: 30 min at	61.5°C	Some survive	45
	17 min at	74°C	Some survive	
1952	Infected milk: 30 min at	63°C	Some survive	46
	1 min at	80°C	Killed	
1956	Infected milk: 30 min at	61.7°C	Some survived	47
	30 min at	62.8°C	Killed	
	15 s at	71.7°C	Killed	
1951	Infected milk: In constant temperature	63°C	30 min	48
	water bath	65°C	<30 min	
1953	Infected milk stored aseptically	—	45 d or >	49
	Infected milk allowed to sour	—	24 h	
1953	In dried cheese made from infected milk	—	30 to 40 d	50
1956	*Coxiella burnetii* in water	20—22°C	160 d or >	51
1957	*Coxiella burnetii* in 10% salt solution	Room temp.	180 d	52
		4°C	270 d	
	Tissues from guinea pig sacrificed at height of fever: Unsalted in glass	4°C	30 d or >	
	In 10% salt solution	4°C	150 d or >	

7.4.2 *The effect of pH*

Some cheeses have a low pH (~ 5.0). It has been suggested that *Coxiella* retains better viability in cheese at neutral pH than at pH 5.0 (pers. comm Robert Heinzen, 24 Jan 2013). This is based on experience of freezing *Coxiella* in acidic media, and also the information in Table 4 showing poor viability in soured milk but viability in dried cheese for 30-40 days. Furthermore, Megaw (1954) summarising a 1953 paper by Combiescu writes that milk collected and maintained in aseptic conditions remained infective for at least 45 days, but if allowed to become sour it ceased to be infective within 24 hours. Unfortunately Megaw (1954) does not give any of the data of Combiescu et al..

7.4.3 *The effect of heat treatment and pasteurisation*

C. burnetii is inactivated by pasteurisation of commercial milk (Tsurumi 2003; Cerf and Condron 2006). Indeed, pasteurisation standards today are based on the destruction of *C. burnetii* (Holsinger et al. 1997). This reflects the resilience of the small cell variant (SCV). There are good data for the effect of pasteurisation of milk (61.7°C to 72.2°C) on inactivation of *C. burnetii* as detected by infection of guinea pigs (Enright et al. 1957).

7.4.4 *The effect of fat content*

There is evidence that the fats and sugars in milk products like cream, chocolate and ice cream mixes may promote survival, such that the pasteurisation conditions defined for whole raw milk are inadequate (Enright 1961). Indeed Enright (1961) recommended that the pasteurisation temperature be increased to kill *C. burnetii* in these products. Thus, if the fat content of the milk product is >10%, or the total solids >18%, or if sweeteners are added, then the specified temperature should be increased by 3°C (Grade A Pasteurised Milk Ordinance, Revision 2009). Kukreja (2011) notes that the composition of the milk products have changed significantly since the 1950s and new products have been launched underpinning the need for development of better detection methods for viable organism in milk products. Data on inactivation by heat treatment of *C. burnetii* in cheese making are lacking (EFSA 2010). However, pasteurisation at 161°F for 15 seconds in cream and chocolate milk is inadequate and 166 F for 15 seconds is recommended (Enright 1961).

7.5 ***Knowledge gaps***

The main knowledge gap is on the degree of inactivation of *C. burnetii* in milk and milk products including different types of cheese. It is well established that the small cell variant of *C. burnetii* is resistant to inactivation. Mike Minnick (Montana University pers comm) is confident that the resistant morphotype predominates in milk because it was the heat resistance of *C. burnetii* which drove the regulations for pasteurisation (Cerf and Condron 2006). Thus long term survival of *C. burnetii* in milk and milk products would be expected. Of interest is the effect of low pH in cheese on survival on the pathogen, and the possibility of greater inactivation in low pH cheeses such as cheddar.

Although there are data on the number of guinea pig ID_{50}s in milk, the risk of infection in man from ingestion of a guinea pig ID_{50} is not known. PCR data, although quantitative gives no information on the viability and it would be useful to know the number of genomic copies which make up a guinea pig ID_{50}. No data have been

found for levels of *C. burnetii* in goat or sheep milk. The genotype of *C. burnetii* may be important in relation to human infection but there is not sufficient information at present to include in the risk profile.

7.6 ***Summary and conclusions***

A number of studies have detected the presence of DNA in milk and cheese by PCR. However, PCR has had a clear disadvantage on obtaining data useful for risk assessment with regard to the viability. In this respect, the guinea pig/mice infection assays undertaken before the advent of PCR offer much greater value for risk assessment (although it is not known how to convert intraperitoneal guinea pig infectious dose 50% units into human oral ID_{50}s). From viability studies in mice, the few available results suggest that *C. burnetii* may be viable in raw milk but not in cheeses made from unpasteurised milk; however it is noted that there are very few studies in this area. While more extensive sampling of cheeses could detect viable bacteria, it is also possible that the combination of time/process conditions (e.g. lower pH) in cheese making are not conducive to survival of the pathogen. Indeed Eldin et al. (2013) "believe that *C. burnetii* does not survive in cheese (and yogurt)". However, viable *C. burnetii* has been demonstrated to survive in cottage cheese for up to 42 days in one study (Sipka 1958).

There are some data from the 1950s on the levels of *C. burnetii* in cows' milk. Furthermore these give information on the viability of the pathogen. However, the units are intraperitoneal quinea pig ID_{50}s, which are difficult to interpret in terms of oral risk to humans. *C. burnetii* is likely to survive in milk for considerable periods of time (i.e. weeks).

8. STUDIES RELATING TO OTHER ROUTES OF TRANSMISSION

As discussed previously, the main route of human infection with *C. burnetii* is considered to be inhalation via direct or indirect contact with the secretions and excretions from infected animals. Many studies have been undertaken to investigate the presence and level of the organism in the placenta (Babudieri 1953; 1959) and its survival capability in the environment (Kersh et al. 2013).

In particular, as discussed above the placenta of an infected animal may contain up to 10^9 organisms/g of tissue and it is likely that heavily infected placentas contaminate the environment at the time of parturition, leading to the persistence of viable organisms in the soil for several months (Langley 1990). Survival in the environment has also been reported as extending to years (Van Woerden et al. 2004). Semi-quantitative PCR data confirm that *C. burnetii* loadings are highest in

placenta tissue and vaginal mucus but very low in milk from goats (Roest et al. 2012). Indeed, the maximum microbial loadings per g of placenta are 100,000-fold higher than per ml of milk.

Voth and Heinzen (2007) report that the organism is highly infectious with an infectious dose approaching one organism. This is the case through inhalation at least, and possibly through the intraperitoneal route. However transmission through the oral route appears to be much less efficient. Indeed, the lower infectivity of this pathogen through the oral route compared to the inhalation route probably reflects the lower numbers of target macrophages in the GI tract compared to the lungs. Thus the lung tissue with a high number of alveolar macrophages is a prime environment for initial infection and the most common route of infection by *C. burnetii* (Mike Minnick, Montana University, pers comm.). Taken together with the fact that the risk of infection from one cell is 90% through inhalation, but lower through the oral route, the difference in exposures would be consistent with milk and milk products being a minor route compared to inhalation of aerosols from livestock birth products.

9. MILK/MILK PRODUCTS ROUTE VERSUS OTHER ROUTES

9.1 ***Evidence for the importance of milk/milk products route compared to other routes***

Epidemiological evidence suggests that the main route of infection of humans with *C. burnetii* is through aerosols from animal birth products or livestock. The reasoning and evidence for this are now summarised:-

1. Infectivity may be lower through the oral route than the inhalation route, perhaps reflecting the smaller numbers of target cells (macrophages) in the gut compared to lung tissue. Thus, although each single organism may have a 90% chance of infecting a human through the inhalation route, available evidence suggests risks through the oral route are lower (Fonseca et al, 1949; Krumbiegel and Wisniewski (1970)). Indeed, Cerf and Condron (2006) even challenge the designation of *C. burnetii* as a foodborne pathogen.

2. The pathogen may be less viable in some milk products compared to others, although there is conflicting evidence. Eldin et al. (2013) conclude that although farm animals are highly infected in France, consumption of cheese and yoghurt does not seem to pose a public health risk for transmission of Q fever because the pathogen is not viable. This may reflect inactivation of the pathogen during the cheese making process together with the fact that the infectivity of the pathogen is lower through ingestion than inhalation as discussed above. However,, the pathogen is clearly viable to some degree in

milk because naturally infected milk has been shown to infect guinea pigs and mice in experiments albeit through intraperitoneal challenge (Bell et al. 1949; Enright et al. 1957; Loftis et al. 2010). Furthermore, viable pathogen has been detected in cottage-type cheese after 42 days in one study (Sipka 1958).

3. The potential exposures to humans through milk/milk products may be lower than through livestock lairage and/or animal birth products. The evidence for this is that huge numbers of bacteria (10^9 per gram) are produced during abortion and via livestock birth products compared to much lower levels in milk. Indeed, the maximum microbial loadings per g of placenta are 100,000-fold higher than per ml of milk. A very recent air sampling study on a goat farm in the USA has shown mean levels of *C. burnetii* DNA to be 98 – 138 genome equivalents per 500 litres of air in areas around the farm one year after the outbreak.

4. Changes at dairy industry level did not have an effect on the seroprevalence for *C. burnetii* in humans in the Netherlands which decreased from 44.0% in 1983 to 2.4% in 2007 (Brandwagt 2012). This is consistent with milk and milk products being a minor pathway for infection of the population with Q fever. Indeed, Maurin and Raoult (1999) in their review of Q fever conclude that although milk may contain large amounts of *C. burnetii*, it is probably a minor route of Q fever acquisition. Maurin and Raoult (1999) go on to write that ingestion (mainly drinking raw milk) is probably a minor factor in the transmission of *C. burnetii* and is now even a point of controversy.

5. There is strong epidemiological evidence for windborne transmission of Q fever from livestock farms to people in nearby towns. In contrast, the epidemiological evidence for transmission through milk is much weaker. Epidemiological evidence shows that contact with farms animals is a major risk factor, while the evidence of infection through consumption of raw milk products is less strong. This is consistent with the exposures being lower through milk products and/or the bacterium being less infectious through milk products compared to aerosolised bacteria from livestock births or abortions.

9.2 ***Knowledge gaps***

The main knowledge gap is the dose-response for *C. burnetii* in milk for humans. While there are relatively good data (albeit from the 1950s) on the number of guinea pig intraperitoneal ID_{50}s in cows' milk, it is not known how many guinea pig intraperitoneal ID_{50}s comprise an oral ID_{50} in humans. It is also not known how infectious *C. burnetii* is to humans through the oral route, although through inhalation the risk from a single organism may be 90%. Suggestions that *C. burnetii* is not viable in milk or milk products are clearly incorrect, since there is considerable evidence to demonstrate infection in animal models. Although there are little

quantitative data on survival in milk, it is assumed that the low temperatures and short time periods do not support inactivation of the pathogen. A second knowledge gap relates to the fate of the pathogen in cheese. First, it is not known how much of the pathogen is removed with the whey. Indeed if *C. burnetii* is “water-soluble”, i.e. does not partition into fat, then some 95% could be removed with the whey. Second there is some evidence that low pH promotes inactivation. Thus the low pH environments of some cheeses could facilitate inactivation of the pathogen. There are no qualitative or quantitative studies on consumption patterns of unpasteurised milk and milk products in GB.

9.3 ***Summary and conclusions***

Overall it is concluded that milk is a minor route for *C. burnetii* transmission to humans compared to other routes such as windborne aerosol transmission from birth products. This is based on two main pathogen-related reasons, namely:-

1. The relatively low loadings of *C. burnetii* in milk compared to placenta; and
2. The lower infectivity of this pathogen through the oral route compared to the inhalation route probably reflecting the lower numbers of target macrophages in the gastrointestinal tract compared to the lungs.

10. **CONCLUSIONS**

The overall conclusion of this work is that the risks of *C. burnetii* infection to humans through consumption of raw milk and milk products (including cheese) are not negligible but they are lower in comparison to transmission via inhalation of aerosols from parturient products and livestock contact. This is thought to be attributable to the relatively low loadings of *C. burnetii* in milk compared to placenta and also the lower infectivity of this pathogen though the oral route compared to the inhalation route. The risks may be lower for certain cheeses than milk, particularly those cheeses with long maturation times and low pH. However, a major source of uncertainty with regard to cheese is the degree of partition of the organism into the curd and hence the proportion which is removed with the whey. While more information on the numbers of viable pathogen in milk and milk products would greatly assist the exposure assessment, it is unlikely there will ever be sufficient dose-response data for the oral route in humans. Even if a foodborne outbreak could be detected, calibration of a dose response would currently not be possible because of the lack of an enumeration method for viable organism.

Due to uncertainty in much of the data, a risk assessment for infection through milk and milk products cannot be undertaken at present. These gaps in knowledge on prevalence are compounded by the experimental obstacles in culturing *C. burnetii* associated with intracellular obligatism (Omsland and Heinsen 2011) and therefore data on prevalence of infection between and within herds, survival of the organism and dose-response are lacking. In this respect, a key recommendation of this work is that methods be developed for detecting and enumerating viable *C. burnetii*.

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