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Q fever through consumption of unpasteurised milk and milk products – a risk profile and exposure assessment

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Abbreviated Title: *Coxiella burnetii* exposures through raw milk

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Keywords: *Coxiella burnetii*, risk assessment, exposure, unpasteurized milk

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Abstract

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii* which is endemic in cattle, sheep and goats in much of the world, including the United Kingdom (UK). There is some epidemiological evidence that a small proportion of cases in the developed world may arise from consumption of unpasteurised milk with less evidence for milk products such as cheese. Long maturation at low pH may give some inactivation in hard cheese and viable *C. burnetii* are rarely detected in unpasteurised cheese compared to unpasteurised milk. Simulations presented here predict that the probability of exposure per person to one or more *C. burnetii* through the daily cumulative consumption of raw milk in the UK is 0.4203. For those positive exposures, the average level of exposure predicted is high at 1,266 guinea pig intraperitoneal infectious dose 50% units ($GP_IP_ID_{50}$) per person per day. However, in the absence of human dose-response data, the case is made that the $GP_IP_ID_{50}$ unit represents a very low risk through the oral route. The available evidence suggests that the risks from *C. burnetii* through consumption of unpasteurised 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.

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INTRODUCTION

Q fever is a widespread, zoonotic disease caused by the bacterium *Coxiella burnetii* which is endemic in livestock including cattle, sheep and goats in much of the world including the United Kingdom (UK) (Maurin and Raoult 1999; Cutler *et al.* 2006). The clinical manifestations of Q fever in humans are variable, ranging from asymptomatic to serious. 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 mortality. 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. Large community outbreaks of Q fever with over 3500 notified cases occurred in the Netherlands between spring 2007 until the end of 2009 (Schimmer *et al.* 2011). The aerosol route (inhalation of infected fomites) is considered to be the primary mode of human infection with *C. burnetii*. Infection via *C. burnetii* aerosols may occur from direct contact with the excretions and secretions from infected animals. These include milk, urine, faeces, vaginal mucus, semen and parturient fluids, which may contaminate newborn animals, placenta, or wool (Maurin and Raoult 1999).

Viable *C. burnetii* can be shed in milk from infected livestock including cattle (Bell *et al.* 1949; Enright *et al.* 1957) and have been detected (by passage in mice) in commercial unpasteurised milk samples (Loftis *et al.* 2010). However, the viability in those milk samples was demonstrated by intraperitoneal challenge rather than oral challenge. Indeed, the link between infection and clinical disease in humans through consumption of unpasteurised milk and milk products is unclear (EFSA 2010). Maurin and Raoult (1999) in their review of Q fever conclude that although milk may

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contain large amounts of *C. burnetii*, it is probably a minor route of Q fever acquisition.

The aim of this paper is to assess the risks of *C. burnetii* infection through consumption of unpasteurised milk and milk products. The paper first reviews the epidemiological evidence for routes of transmission to humans and then addresses the feasibility of developing a quantitative risk assessment for milk and milk products. The availability of data limits this study to predicting human exposures to viable *C. burnetii* through consumption of unpasteurised cows' milk. The risks from these exposures are interpreted on the basis of infectivity through the oral route and placed in context against the risks through other routes of transmission, in particular inhalation of aerosols from livestock birth products. The potential risks of unpasteurised milk products, namely cheeses, relative to milk are also considered.

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EPIDEMIOLOGICAL DATA ON ROUTES OF TRANSMISSION OF *C. BURNETII* TO HUMANS

There are a number of routes identified by epidemiological studies for transmission of *C. burnetii* to humans.

Aerosols and direct contact with livestock

The main routes of transmission are from livestock and companion mammals either through the environment or through direct contact (Langley *et al.* 1988; Connolly *et al.* 1990; Thomas *et al.* 1995; Schimmer *et al.* 2011). In this respect aerosolisation and inhalation appear to be important (Maurin and Raoult 1999). Indeed outbreaks associated with windborne transmission from farms and slaughter houses and within meat processing plants are well-documented (Brouqui *et al.* 2004; Tissot-Dupont *et al.* 2004; Wilson *et al.* 2010). The resistance of *C. burnetii* promotes its transmission through aerosols, and there are suggestions of outbreaks of Q fever arising from *C.*

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burnetii sources many years after release from an infected host (van Woerden *et al.* 2004).

Consumption of unpasteurised milk and milk products

There is epidemiological evidence, from the developed world, that cases of Q fever have occurred where consumption of unpasteurised milk was the most likely cause. The most recent of these was in Michigan (USA) in 2011 and involved five individuals (Signs *et al.* 2012). However, suspected milk borne outbreaks are rare in the UK. Unpasteurised cows' milk purchased by a patient was thought to be responsible for an outbreak of Q fever in a London hospital in 1950 (Marmion and Harvey 1956) and it was concluded that raw milk was responsible for an outbreak of Q fever in a boys' detention centre in Staffordshire in April 1967 (Brown *et al.* 1968). Although these studies are highly suggestive of the consumption of unpasteurised milk being the source of the outbreak, there is still uncertainty associated with this link (EFSA 2010). An epidemiological study of Q fever cases in the UK from 1984-1994 has reported that, out of 1,117 cases of Q fever investigated, three cases were reported to have consumed unpasteurised milk (Pebody *et al.* 1996). With the possible exception of an outbreak in France (Raoult *et al.* 2000) where unpasteurised 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.

PROPERTIES OF THE *C. BURNETII* ORGANISM WITH RELEVANCE TO ASSESSING THE RISKS THROUGH UNPASTEURISED MILK AND MILK PRODUCTS

Infectious *C. burnetii* have been isolated from milk

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There is much evidence that *C. burnetii* is viable to some degree in unpasteurised milk. Thus, Loftis *et al.* (2010) have confirmed the viability by passage in mice of *C. burnetii* in at least two and maybe four of six PCR-positive commercial, unpasteurised milk samples. Experiments conducted in the 1940s and 1950s showed that naturally infected cows' milk can infect guinea pigs and mice (Bell *et al.* 1949; Enright *et al.* 1957) albeit through intraperitoneal challenge. Levels of viable *C. burnetii* in milk are often expressed in units of "guinea pig intraperitoneal infectious dose 50%" or $GP_IP_ID_{50}$. This is the dose which, when given to each and every member of a group of guinea pigs through intraperitoneal challenge, results in 50% being infected (Enright *et al.* 1957).

***C. burnetii* will not multiply in the milk**

C. burnetii is an obligate intracellular bacterium that relies exclusively on a eukaryotic cell for growth (Omsland and Heinzen 2011). This has direct relevance to assessing the risks through food and environmental routes because *C. burnetii* does not grow outside the intracellular environment of the host cell. Thus for the purpose of risk assessment it is assumed that multiplication of the pathogen in milk and milk products does not occur.

The environmental morphotype is highly resistant

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) (Minnick and Reghavan 2012). Unlike other obligate intracellular bacteria, *C burnetii* has spore-like environmental stability due to the resistance of the SCV (Oyston and Davies 2011). Indeed *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 (Oyston and Davies

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3 2011). Monocytes and macrophages are the major targets of *C. burnetii* (Amara *et al.*
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5 2012) and spread around much of the body. The placenta is a tissue rich in
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7 macrophages and placental macrophages harbour *C. burnetii* (Amara *et al.* 2012).
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10 **The unit of *C. burnetii* infectivity in milk**
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12 Macrophages occur in bovine milk (Paape *et al.* 2003). Within the macrophage, a high
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14 density mixture of LCVs and SCVs exists in the parasitophorous vacuole (Minnick
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16 and Reghavan 2011). The LCV is very fragile (Minnick and Reghavan 2011). While
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18 the long survival of *C. burnetii* infectivity in milk (Combiesco *et al.* 1953; Zubkova
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21 1957) supports the case for SCVs being present in milk, there is no information on the
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23 relative proportions of LCV to SCV in macrophages in fresh milk. PCR would detect
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25 DNA from both SCVs and LCVs in milk, with the SCV representing a higher risk to
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27 human health due to its greater chance of surviving not only in the milk environment
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29 but also in the digestive tract during initiation of infection after consumption of
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32 infected milk.
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34 *Estimating the number of C. burnetii bacteria comprising a GP_IP_ID_{50} in milk.*
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36 Ideally the exposure units for a quantitative risk assessment should be in terms of the
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38 number of viable bacteria such that the outputs can be used directly in a dose-response
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41 model should one become available (see below). Thus, expressing *C. burnetii*
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43 exposures in terms of the numbers of GP_IP_ID_{50} raises the question of how many *C.*
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45 *burnetii* bacteria comprise GP_IP_ID_{50}. Guatteo *et al.* (2007) used a PCR method to
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47 estimate titres in cows' milk by comparison of PCR results with those from solutions
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50 with a known *C. burnetii* concentration obtained by serial dilution of an external
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52 positive control. Comparison of quantitative PCR results of Guatteo *et al.* (2007) for
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54 *C. burnetii* in dairy milk with the numbers of GP_IP_ID_{50} recorded in milk by Enright
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56 *et al.* (1957) suggest there could be between 2 and 112 *C. burnetii* organisms per
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3 GP_IP_ID$_{50}$ in milk. This is calculated as follows. The distribution of GP_IP_ID$_{50}$ in milk from 18 naturally infected and shedding dairy cows appears to be log-Normal with a mean of 98.75 per ml (Enright *et al.* 1957). The averaged median and averaged maximum (for n = 5 cows) number of *C. burnetii* per ml of milk (quantified by PCR in Guatteo *et al.* (2007)) were 213 and 11,073, respectively. Since the mean of a log-Normal distribution is between the median and the maximum, the mean number of *C. burnetii* is between 213 and 11,073 per ml of milk which is equivalent to 98.75 GP_IP_ID$_{50}$. This suggests there are between (213/98.75) 2 and (11,073/98.75) 112 *C. burnetii* organisms per GP_IP_ID$_{50}$ in milk.

FEASIBILITY OF DEVELOPING A QUANTITATIVE RISK ASSESSMENT FOR *C. BURNETII* INFECTION THROUGH MILK AND MILK PRODUCTS

Milk products include cheese, yoghurt, butter and cream. Milk and milk products may be sourced from cattle, sheep and goats. Thus data are needed for each of these species although in terms of consumption patterns, the use of cows' and goats' milk is more common than for sheep's milk and unpasteurised cheese and yoghurt are normally made from cows' or goats' milk in England.

Availability of dose-response data for infection of *C. burnetii* through the oral route in humans

C. burnetii is highly infectious through inhalation with the risk of infection from a single bacterium estimated to be as high as 0.9 in guinea pigs (Jones *et al.* 2006). There are insufficient data for a dose-response model for the oral route in humans. Indeed, transmission by the oral route of *C. burnetii* is controversial (Eldin *et al.* 2013) and Cerf and Condron (2006) challenge the designation of *C. burnetii* as a foodborne pathogen. This suggests *C. burnetii* may not be very infectious through the oral route. Over a period of one month, Krumbieoel and Wisniewski (1970) gave 34

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volunteers unpasteurised milk that was naturally infected with *C. burnetii*. The volunteers consumed an average of 4.5 litres of milk under supervision during the month of the trial. None of the volunteers developed any clinical symptoms even after 12 years and serum samples taken 1 month and 2 months after initial ingestion showed no evidence of seroconversion. 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. Two of 11 patients in an asylum in Portugal given *C. burnetii* in food showed signs of seroconversion by complement fixation assay and none developed clinical symptoms (Fonseca *et al*, 1949). The doses administered in that study were not specified and it is unlikely there will ever be sufficient dose-response data for *C. burnetii* infection in humans through the oral route to undertake a quantitative risk assessment. Even if a foodborne outbreak could be detected, calibration of a dose-response would currently be difficult because of the lack of a straightforward enumeration method for viable organism.

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Availability of data for estimation of exposure through consumption of unpasteurised milk

The data required for predicting levels of exposure through consumption of unpasteurised milk are set out in the exposure pathway in Figure 1.

Prevalence of C. burnetii in livestock in UK. C. burnetii is endemic in UK dairy cattle herds which, in the case of dairy herds in Northern Ireland at least, have higher prevalences than beef cattle herds (McCaughey *et al.* 2010). Reported prevalences in bulk tank milk (BTM) samples from dairy cattle herds in England and Wales range from 22% (ELISA) to 69.7% (PCR) (Paiba *et al.* 1999; Valergakis *et al.* 2012). McCaughey *et al.* (2010) present data for between herd and within herd prevalence according to herd size. There are fewer data for sheep and goats in England and Wales

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with unpublished estimates of individual animal prevalences in sheep and goats of 0.92% and 0.78%, respectively, by ELISA (Lambton *et al.* unpublished) although data have been published for Northern Ireland (McCaughey *et al.* 2010). The advent of PCR has enabled detection of *C. burnetii* DNA and even quantification of *C. burnetii* DNA in milk as used by Valergakis *et al.* (2012) for BTM from dairy cattle in south-west England. However, the problem with PCR is that it gives no information on the viability of the organism. 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, but not actively infected with the bacteria, although some may later convert from sero-positive to sero-negative.

Probability of infected livestock shedding C. burnetii. Shedding of *C. burnetii* differs among ruminant species, milk being the primary route of shedding in cattle and goats (Rodolakis *et al*. 2007). Sheep shed mainly in the faeces and vaginal mucus and to a lesser extent in milk (Rodolakis *et al*. 2007). Indeed, for infected goats, 31 – 38% shed in milk (Rousset *et al.* 2009). Roest *et al.* (2012) reported that all *Coxiella*-inoculated goats excreted *C. burnetii* DNA in milk post partum. Guatteo *et al.* (2012) give data on the number of infected cows which were shedding at days 14, 21 and 28 post abortion due to *C. burnetii*.

Levels of viable C. burnetii in milk of shedding livestock. Enright *et al.* (1957) used a guinea pig bioassay approach to measure *C. burnetii* in unpasteurised cows' milk. The great advantage (for the purpose of data for risk assessment) of guinea pig bioassay over PCR is that it determines viable pathogen in terms of the numbers of $GP_IP_ID_{50}$. Enright *et al.* (1957) reported that milk from 18 of 137 individual cows in a naturally-infected dairy herd contained viable *C. burnetii*. Titration of those positive milk samples showed levels of 1,000 (n = 3), 100 (n = 5), 10 (n = 5) and one

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(n = 5) GP_IP_ID$_{50}$ per 2 ml. The mean number of *C. burnetii* is therefore 98.8 GP_IP_ID$_{50}$ per ml of milk from a shedding cow. Bell *et al*. (1949) reported a maximum of 10^5 GP_IP_ID$_{50}$ (presumably per ml) in milk from a cow with mastitis. This value is excluded from the analysis here because the mastitis may have increased the measured densities of *C. burnetii* in milk by two mechanisms; namely i) by increasing the number of *C. burnetii*-infected macrophages actually in the milk (Paape *et al*. 2003) and ii) by decreasing the volume of milk produced. From a risk assessment perspective, the milk from cows with mastitis would not enter the food chain. Similarly data, including a maximum of 10,000 GP_IP_ID$_{50}$ per 2 ml of milk, recorded from a dairy cow (Enright *et al.* 1957) were excluded here because that cow was experimentally (as opposed to naturally) infected by introduction via the teat canal.

There are no quantitative data on levels of viable *C. burnetii* in sheep and goats' milk.

Duration of shedding in milk. Shedding of *C. burnetii* DNA in milk from infected goats stopped 38 days post partum (Roest *et al.* 2012), although Arricau-Bouvery *et al.* (2003) detected *C. burnetii* DNA in goats' milk 52 days after abortion. Guatteo *et al.* (2012) write that three infected cows were identified as persistent shedders in that they were shedding relatively high levels at 14, 21 and 28 days post abortion. Unfortunately Guatteo *et al.* (2012) do not give data for more than two weeks (albeit one month after abortion). Enright *et al.* (1957) give data showing that infected cattle can shed in milk for periods of more than one year. They found that the milk of four positive cows was still positive 205 days after each had calved, and one of the animals was found to be still shedding 1,000 GP_IP_ID$_{50}$ per 2 ml of milk. Serologic evidence indicated that this animal was infected at least 405 days prior to the second milk

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sampling. *C. burnetii* could not be found in the milk of the other three cows at the time point of the second milk sampling.

Levels of consumption of unpasteurised milk in the UK. There are no data available on consumption of unpasteurised milk in the UK. The mean consumption for milk has been estimated as 0.127 kg/person/day (Department of Health 2011). This is for (pasteurised presumably) whole milk (3.8% fat) among the 19 to 64 year old age group, and includes males and females and, importantly, consumers only.

Availability of data for estimation of exposure through consumption of unpasteurised milk products

Recently some papers have been published reporting results of PCR studies for detection of *C. burnetii* DNA in unpasteurised cheeses. As an example, Capuano *et al.* (2012) reported 21.3% of cheeses made in Southern Italy from unpasteurised milk were PCR-positive. Hirai *et al.* (2012) reported 7 of 41 commercial cheeses made from unpasteurised milk were PCR-positive, compared to 20 of 96 made from pasteurised milk. To date, however, no published paper has been found giving counts of viable *C. burnetii* in cheese with which to compare with the unpasteurised milk data of Enright *et al.* (1957).

Proportion of C. burnetii removed with the whey during cheese-making. Removal of the whey during cheese production could eliminate a considerable proportion of the pathogen, although there are no specific data for *C. burnetii* in this respect. Anon (2013) present the relative proportions of milk components that remain in the whey or partition into the cheese. The data show there are two exclusive outcomes. Thus, around 95% of the water soluble components (namely water, lactose and non-precipitated proteins) remain in the whey and are removed with the whey, while 95% of the water-insoluble components, namely fat and precipitated casein proteins go into

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the cheese. Thus it could be that either 95% of the *C. burnetii* are lost with the whey, or alternatively that 95% of the *C. burnetii* precipitate into the curds which go on to become cheese. The amount of whey is to some extent affected by salt content and impacts on moisture content of the cheese which differ between soft and hard cheeses. For *C. burnetii*, this could be addressed by considering the physical properties of the small cell variant at low pH or after rennet treatment.

Survival of C. burnetii in milk and milk products with time. Much of the data on *C. burnetii* survival was obtained from experiments in the 1940s and 1950s. Although *C. burnetii* is inactivated by pasteurisation, there is little evidence that any of the processes used for unpasteurised cheese, cream or butter production would significantly inactivate *C. burnetii*. Jellison *et al*. (1948) reported the presence and persistence of infectious *C. burnetii* in butter made from naturally infected milk and *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). There is one study where viable pathogen has been detected in a cottage-type cheese after 42 days (Sipka 1958). The data are not quantitative and inactivation rates cannot be determined.

Effect of low pH. Based on experience of freezing *Coxiella* in acidic media it is believed that *Coxiella* may retain better viability in cheese at neutral pH than at pH 5.0 (Robert Heinzen, National Institute of Health, USA, pers. comm.). This is supported by data from the 1950s that milk collected and maintained in aseptic conditions remained infective for at least 45 days, but if allowed to become sour (lower pH) it ceased to be infective within 24 hours (Combiesco *et al.* 1953).

Summary of identified data gaps

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There are significant data gaps in the level of knowledge of *C. burnetii* with little or no information on:-

1. Current farm prevalence and within herd/flock prevalence of *C. burnetii* (ELISA and PCR data are available but will overestimate the prevalence);
2. Levels and viability of *C. burnetii* in sheep and goats' milk;
3. Survival of *C. burnetii* in unpasteurised milk and milk products;
4. Survival and removal of *C. burnetii* during the cheese-making processes and manufacture of other milk products; and
5. Dose-response data for humans through the oral route.

The data gaps in part reflect the difficulties in routine culture of *C. burnetii* (Oyston and Davies 2011) and also the lack of data on the viability of the organisms when DNA is detected by PCR methods. It is concluded that there are insufficient data to develop a quantitative risk assessment for *C. burnetii* in sheep and goats' milks, or in milk products including cheese. There are, however, sufficient data to predict exposures of *C. burnetii* (albeit in terms of $GP_IP_ID_{50}$) through consumption of unpasteurised cows' milk and this is now described.

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A QUANTITATIVE EXPOSURE ASSESSMENT FOR CONSUMPTION OF UNPASTEURISED COWS' MILK

The specific question that the exposure assessment will address is, "*What is the exposure to C. burnetii of a consumer through the cumulative consumption of unpasteurised cows' milk over the period of one day?*". This may be broken down into two outputs, namely:-

1. The probability of exposure through the cumulative daily consumption of unpasteurised milk; and

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2. The level of exposure, given exposure has occurred, to a person through consumption of unpasteurised milk over the period of a day.

The exposure pathway is shown in Figure 1. The model parameters, based on the data described previously are given in Table 1. The between-herd and within-herd prevalences used are those for Northern Ireland (McCaughey *et al.* 2010) and are broken down according to herd size. It is assumed that the probability that an infected cow is shedding ($p_{Shedding}$) is given by 22/72 (0.3055) according to the summed data of Guatteo *et al.* (2012) over days 14, 21 and 28 post abortion due to *C. burnetii*. As a worst case, it is assumed that a cow which does shed *C. burnetii* does so for the whole year. A Normal distribution gave a good fit (χ^2 = 0.667,1 df; P = 0.88) to the log_{10}-transformed titres for GP_IP_ID_{50}/ml milk from shedding cows and was used in the risk assessment.

The quantitative model was implemented in Microsoft Excel, using the @RISK software package to incorporate variation associated with herds and individual animals in relation to infection, lactation and the levels of *C. burnetii* in milk. There are no quantitative data to allow estimation of a decay rate for *C. burnetii* in milk and it is assumed that no decay occurs between milking and consumption of fresh milk.

The predicted mean level of *C. burnetii* in unpasteurised cows' bulk tank milk (per herd) in the UK

The model simulated each cow in a herd on a given day and each iteration of the model represents the milk produced from a single herd on that day. In total 500,000 iterations were run representing 500,000 herd-days. For each iteration, the number of cows (H) in the herd was randomly selected from the empirical distribution of herd sizes for the 81 herds in England and Wales known to be producing unpasteurised milk (data provided by UK Food Standards Agency). Taking into account the

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3 between-herd prevalence (p_{Herd}), the within herd prevalence (p_{Within_herd}), the
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5 probability of lactating ($p_{Lactating}$) and the probability of shedding given infection
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7 ($p_{Shedding}$) (Table 1), binomial distributions were used to simulate whether or not each
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10 cow in the herd was producing infected milk on that day. For each shedding cow the
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12 number of *C. burnetii* $GP_IP_ID_{50}$s (C_{day}) contributed to the BTM on that day was
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14 calculated as the product of the volume of milk produced by that cow on that day (V)
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16 and the concentration (C_{ml}) of infectivity in the milk as drawn from a log-Normal
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18 distribution fitted to the data of Enright *et al.* (1957). The total volume of milk in the
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21 BTM was calculated as the sum of the volumes of milk (V) produced by all lactating
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23 cows in the herd on that day. From the total *C. burnetii* shed from all cows ($C_{BTM,Day}$)
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25 and the total volume of milk produced by the herd, the mean level of *C. burnetii* in the
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27 bulk tank milk ($C_{BTM,Litre}$) from the given herd on a given day was calculated.
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30 Although there will be variation between the individual cows within the herd in the
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32 amount of *C. burnetii* shed each day, the mean is appropriate here because the milk in
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34 the bulk tank is stirred, and furthermore is not mixed with milk from other cattle
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36 herds. This is because of restrictions in England on the sale of unpasteurised cows'
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39 milk (Anon 2006). The simulated mean level ($C_{BTM,Litre}$) of *C. burnetii* is 4,189
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41 $GP_IP_ID_{50}$ per litre of unpasteurised milk from the bulk tank with 2.5^{th} and 97.5^{th}
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43 percentiles of 0 and 26,848 $GP_IP_ID_{50}$ per litre, respectively. This represents the
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45 mean for the 81 unpasteurised milk-producing herds in England and Wales.
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48 *Validation of predicted levels of infectivity in unpasteurised milk against published*
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50 *PCR data.* The seemingly high values predicted for infectivity levels in BTM reflect
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52 the values of up to 1,000 $GP_IP_ID_{50}$s per 2 ml of unpasteurised milk (Enright *et al.*
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54 1957) to which the log-Normal distribution, used in the simulation here, was fitted.
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57 The distribution for the number of *C. burnetii* $GP_IP_ID_{50}$ per ml of BTM milk as
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simulated is presented in Figure 2. The $GP_IP_ID_{50}$s per ml are converted to logarithms to enable direct comparison with the distribution presented in Valergakis *et al.* (2012) of the qPCR units per ml of milk. The two distributions are similar *in shape* with each having two peaks. The "negative samples" peak reflects negative herds and also positive herds with non-shedding cows on that day. However, although the shapes of the distributions have some similarity, the simulated *C. burnetii* $GP_IP_ID_{50}$ values are shifted by some three logs lower compared to the qPCR data of Valergakis *et al.* (2012). The arithmetic mean number of qPCR units in the BTM of Valergakis *et al.* (2012) is estimated to be 7.36 x 10^6 per litre and 1,800-fold higher than the simulated mean level of 4,189 $GP_IP_ID_{50}$ per litre. Thus the model would appear to underestimate the levels of *C. burnetii* in BTM by some three orders of magnitude compared to PCR data obtained from BTM in the south-west of England. However, there are three considerations which could account for this discrepancy:-

1. The PCR primers used by Valergakis *et al.* (2012) target a sequence of DNA that is present in multiple copies in each *C. burnetii* organism. Thus Klee *et al.* (2006) report 23 IS1111 elements in the genome of the Nine Mile strain, although the number varied between seven and 110 in other isolates;
2. Some of the DNA detected by the PCR may represent non-viable (dead) *C. burnetii* organisms; and
3. A $GP_IP_ID_{50}$ from milk may comprise more than one bacterium such that multiple *C. burnetii* genomes are present in a $GP_IP_ID_{50}$. As discussed above, it is estimated here that there are between 2 and 112 *C. burnetii* organisms per $GP_IP_ID_{50}$ in milk.

It is concluded, therefore, that the predicted $GP_IP_ID_{50}$ in BTM (Figure 2) are not inconsistent with the published qPCR data for BTM (Valergakis *et al.* 2012). Thus if

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each $GP_IP_ID_{50}$ comprised 50 bacteria each with 20 copies of the PCR target sequence (Klee *et al.* 2006), then the number of PCR copies would be 1,000-fold the number of $GP_IP_ID_{50}$. This could account for the differences in the predicted number of $GP_IP_ID_{50}$ per ml of milk (Figure 2) and observed number of PCR copies/ml (Valergakis *et al.* 2012).

The probability and level of human exposure to *C. burnetii* due to the consumption of unpasteurised cows' milk

Exposures were drawn from a Poisson distribution with a mean of the product of the simulated $GP_IP_ID_{50}$ per litre of unpasteurised milk ($C_{BTM,Litre}$) and the amount of milk (0.127 kg) consumed per person per day ($M_{Litre/Day}$). The exposure assessment predicted that the probability of exposure to viable *C. burnetii* , i.e. one or more $GP_IP_ID_{50}$, through the consumption of unpasteurised milk in the UK is 0.4203 per person per day and that the daily exposures, to those who are exposed, will be relatively high with a mean 1,266 $GP_IP_ID_{50}$ per person day and 2.5^{th} and 97.5^{th} percentiles of 2 and 7,524 $GP_IP_ID_{50}$ per person per day, respectively. The magnitudes of these exposures may be over-estimated for three reasons which relate to whether an infected animal is shedding on a given day:-

1. Duration of shedding. It is assumed that an infected cow which is shedding in milk does so every day.
2. Use of serological data (ELISA) for between herd and within herd prevalences may overestimate the proportion of animals infected at any given time.
3. Use of PCR data to estimate the probability of shedding by a cow that experienced abortion due to *C. burnetii* (Guatteo *et al.* 2012) assumes that all *C. burnetii* DNA in milk from an infected cow does indeed represent viable *C. burnetii*.

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In a sensitivity analysis the duration of shedding was reduced to one month (from one year). This decreased the probability of exposure by four-fold to 0.1048 per person per day and decreased the mean level of exposure in those who were exposed by three-fold to 411.5 GP_IP_ID_{50} per person per day (2.5th and 97.5th percentiles of 1 and 2,290 GP_IP_ID_{50} per person per day, respectively)..

ASSESSING THE RISK OF INFECTION THROUGH CONSUMPTION OF UNPASTEURISED COWS' MILK

The predictions here suggest that consumers of unpasteurised cows' milk are frequently exposed to relatively high loadings of *C. burnetii*. Although it is not known how to convert GP_IP_ID_{50} units into human oral ID_{50}s (because of lack of human oral dose-response data), it is likely that each one presents a low risk to humans through the oral route. There are three lines of evidence that support this. These reflect the route of infection, the mechanism of infection and the genotype. First, with respect to the route of infection, Fonseca *et al.* (1949) demonstrated high infection rates by *C. burnetii* in humans through intradermal challenge but low rates through oral challenge (although it is not known if the challenge doses were the same). Intraperitoneal challenge is similar to intradermal challenge and thus it may be argued on the basis of the data of Fonseca *et al.* (1949) that a GP_IP_ID_{50} presents a low risk through the oral route (since 2 of 11 humans were infected by oral challenge compared to 29 of 29 humans by intradermal in Fonseca *et al.* (1949)). Second, with respect to the mechanism of infection, *C. burnetii* targets macrophages within the host tissues in the infection process (Amara *et al.* 2012) and there are far fewer macrophages in the gastrointestinal tract compared to the lung. 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,

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Montana University, personal communication). Thus, *C. burnetii* is less infectious through the oral route compared to inhalation. Third, the genotype of *C. burnetii* may be important in relation to human infection. The genotypes of *C. burnetii* found in a study of commercially available cows' milk in Europe are similar with a dominant genotype that is only incidentally found in humans thus suggesting that the risk of obtaining Q fever via exposure to infected cattle may be much lower than via exposure to infected small ruminants (Tilburg *et al.* 2012). Indeed, sequencing work at AHVLA (Richard Ellis, AHVLA, personal communication) shows that sheep isolates of Q fever are most closely related to those in humans. This is important as sheep shed *C. burnetii* to a lesser extent in milk (Rodolakis *et al.* 2007) and there is little sheep milk consumption in the UK.

RISKS OF INFECTION THROUGH UNPASTEURISED MILK COMPARED TO OTHER MILK PRODUCTS, NAMELY CHEESE

The risks through unpasteurised cheeses may be lower than those for unpasteurised milk. Eldin *et al.* (2013) conclude that although there is a high prevalence of infection in farm animals in France, consumption of cheese does not seem to pose a public health risk for transmission of *C. burnetii* because the pathogen is not viable. This may reflect inactivation of the pathogen in some cheeses. Indeed, the viability of *C. burnetii* appears to be lost in cheese with Hirai *et al*. (2012) reporting no viable *C. burnetii* in 7 unpasteurised milk cheeses which were PCR-positive. However, *C. burnetii* infectivity for guinea pigs was retained in a cottage-type cheese for a period of observation of 42 days (Sipka 1958). Typically, the pH of cheese ranges from 5.1 to 5.9 with a few exceptions such as Camembert which has a pH of 7.44 (World's Healthiest Foods 2013). The pH of cheddar cheese is 5.0 to 5.2 with >60 days' maturation (often 6 to 24 months) (Banks 2006). Semi-soft cheeses such as Caerphilly

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have pH values of 4.6 – 6.2 with 10-14 days' maturation (Banks 2006). It is possible that the combination of time/process conditions (e.g. lower pH and longer maturation times) in the manufacture of some hard cheeses is not conducive to survival of *C. burnetii*. This is consistent with viable *C. burnetii* rarely being detected in unpasteurised cheese (Hirai *et al.* 2012) compared to unpasteurised milk (Enright *et al.* 1957; Loftis *et al.* 2010) and with stronger epidemiological evidence for human cases through unpasteurised milk compared to unpasteurised cheese.

RISKS OF INFECTIONTHROUGH MILK AND MILK PRODUCTS COMPARED TO OTHER ROUTES

Inhalation of aerosols from parturient fluids of infected animals is the primary mode of transmission of *C. burnetii* to humans while ingestion (mainly through drinking unpasteurised milk) is probably a minor factor in the transmission and is now even a point of controversy (Maurin and Raoult 1999; Cerf and Condron 2006). This may reflect a combination of lower exposures and lower infectivity through unpasteurised milk, as is now discussed.

Relative infectivity of *C. burnetii* from oral consumption of milk compared to inhalation of aerosols from birth products.

The *C. burnetii* bacterium may be less infectious through unpasteurised milk compared to aerosolised bacteria from livestock births or abortions because, as discussed above, *C. burnetii* is less infectious through the oral route compared to inhalation reflecting the greater numbers of target macrophages in the lung. In addition it is proposed here that, in terms of tissue origin, *C. burnetii* derived from birth product tissue may be more infectious (on average per bacterium or genome equivalent) through a given route (e.g. intraperitoneal challenge) than that derived from milk. Thus it is estimated above that there may be between 2 and 112 *C. burnetii*

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3 organisms per $GP_IP_ID_{50}$ in milk. In contrast, there is considerable evidence that a
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5 $GP_IP_ID_{50}$ from the placenta comprises just one *C. burnetii* organism. Thus Kersh *et*
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7 *al.* (2013) recorded 1.5 to 2.5 x 10^8 genome equivalents per gram of placenta from
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9 goats and Hansen *et al.* (2011) reported 10^9 *icd* gene copies (single copy per
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11 bacterium) per ml of eluate from cattle cotyledons in parturient cattle. These values
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13 agree well with the average of 5.0 x 10^8 $GP_IP_ID_{50}$ per gram of ovine placental
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15 tissue (Welsh *et al.* 1951). Further experimental work is needed to confirm the
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17 number of *C. burnetii* bacteria in a $GP_IP_ID_{50}$ from milk. The argument presented
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19 here that there are between 2 and 112 bacteria per $GP_IP_ID_{50}$ from milk hinges on
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21 the maximum of 1,000 $GP_IP_ID_{50}$ per 2 ml of milk as recorded by Enright *et al.*
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23 (1957).
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25 It would also be of interest to know whether the ratio of SCV to LCV is the same in
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27 birth products as in milk. Significant differences would affect whether a *C. burnetii*
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29 organism (as represented by a genome equivalent or single bacterium) in milk is as
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31 infectious, on average, as one from birth products, for example. These are important
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33 considerations for developing any risk assessment to compare risks through milk and
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35 aerosolised birth products, particularly since relatively few PCR-based studies address
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37 the viability in milk (Loftis *et al.* 2010).
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39 **Relative exposures through birth products compared to unpasteurised milk**
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41 The exposures to humans may be lower through consumption of unpasteurised milk
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43 than through aerosols from birth products. Huge numbers of bacteria are produced
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45 during abortion caused by *C. burnetii* and via livestock birth products (10^9
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47 $GP_IP_ID_{50}$s per gram of sheep placenta (Welsh *et al.* 1951)) compared to the mean
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49 of 98.8 $GP_IP_ID_{50}$s per ml of unpasteurised milk from shedding cows. Roest *et al.*
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51 (2012) give semi-quantitative PCR data on excretion of *C. burnetii* in goats' milk,
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demonstrating very low levels compared to goat placental tissue. A recent air sampling study (Kersh *et al.* 2013) on a goat farm in the USA has shown the mean level of *C. burnetii* DNA (n = 30) to be 98 genome equivalents per 500 litres of air in areas around the farm one year after an outbreak. The lung tidal volume for a person is approximately 0.5 litre per breath, and a farm worker taking 15 breaths per minute over an 8 h working day would inhale 3,600 litres which would equate to a mean of 706 *C. burnetii* genome equivalents per person per working day. Mean levels of *C. burnetii* DNA were 4.6-fold higher on the farm during the outbreak compared to a year later when the air sampling was undertaken (Kersh *et al.* 2013). Thus exposures on the farm during the outbreak through inhalation may be at the level of >3,000 *C. burnetii* genome equivalents per person per working day and considerably higher than the 532 GP_IP_ID$_{50}$ per person day predicted above through consumption of unpasteurised milk. Without knowing how many bacteria there are in a GP_IP_ID$_{50}$ in milk it is not possible to compare directly exposures through inhalation with those through consumption of unpasteurised milk. In relation to exposure to *C. burnetii* due to contact with birth products, farmers, vets and abattoir workers are most at risk. During lambing season, in particular, exposure to such products will increase and therefore to mitigate this risk (and that of acquiring other zoonotic pathogens), pregnant women are advised to avoid close contact with sheep (NHS, 2014). No information is available on the numbers of consumers that drink raw milk.

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DISCUSSION

A quantitative risk assessment for transmission of *C. burnetii* to humans through milk and milk products is not feasible at present because much of the data required are missing. For example, while there are data from the 1950s on the number of

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$GP_IP_ID_{50}$ units per ml of unpasteurised milk, there are no dose-response data to relate how infectious a $GP_IP_ID_{50}$ unit is to humans through the oral route and there are no quantitative data on survival in milk or milk products over time. *C. burnetii* is viable in naturally infected unpasteurised milk. Thus it is well documented that guinea pigs and mice have been experimentally infected albeit through intraperitoneal challenge (Bell *et al.* 1949; Enright *et al.* 1957; Loftis *et al.* 2010).

Using the data of Enright *et al.* (1957) on levels of viable *C. burnetii* in milk of shedding cows, it has been possible here to model the daily exposures to consumers of unpasteurised cows' milk. The simulations demonstrate that daily exposures to viable *C. burnetii* (in terms of $GP_IP_ID_{50}$) per person through unpasteurised milk are high. This is consistent with recently published data from qPCR studies on cow BTM samples taken in south-west England. This raises the question of how infectious *C. burnetii* in milk is to humans through the oral route. Several lines of evidence are presented here that these predicted high daily exposures through consumption of unpasteurised milk present a relatively low risk to public health. There is little information on the amount of milk which is consumed unpasteurised in England and Wales, although the proportion is likely to be small. Thus, on the basis that there are 7,011 cows in unpasteurised-milk producing herds in England and Wales (data provided by UK Food Standards Agency) and 2,864,000 dairy cows in England and Wales (Helen Gartner, AHVLA, personal communication), it may be estimated that just 0.24% of the total cows' milk is consumed unpasteurised.

Although some authors have gone as far as challenging the designation of *C. burnetii* as a foodborne pathogen, it is concluded here that the risks to humans from *C. burnetii* through consumption of unpasteurised milk and milk products (including cheese) are not negligible but they are lower in comparison to transmission via

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inhalation of aerosols from parturient products and livestock contact. This reflects the lower risk of infection of *C. burnetii* through the oral route compared to the inhalation route and also the much higher loadings in birth products compared to milk, potentially giving higher exposures across the population through aerosols. There is also some tentative evidence presented here to suggest that the pathogen is less infectious in milk than in placentas (per DNA copy), although this needs further substantiation.

C. burnetii has spore-like environmental stability due to the resistant SCV morphotype which probably exists in milk and accounts for the survival of infectivity in milk and milk products over long periods. While there are no obvious barriers in the manufacturing of milk products, the risks may be lower for certain cheeses than milk, particularly those cheeses with long maturation times at low pH. This is consistent with viable *C. burnetii* rarely being detected in unpasteurised cheese compared to unpasteurised milk and with stronger epidemiological evidence for human cases through unpasteurised milk compared to unpasteurised cheese. A major source of uncertainty with regard to cheese is the degree of partition of the organism into the curds and hence the proportion which 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, reducing the level of exposure by 20-fold. Future studies could involve using qPCR to estimate levels of *C. burnetii* DNA in the whey and curds.

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Table 1: Summary of data used for estimating probability and levels of *C. burnetii* in BTM (per herd) for cattle in the baseline model.

Description	**Parameter**	**Summary of data probability distribution**	**Reference**
Number of dairy cows per herd	H	Used empirical data for 81 cattle herds in England and Wales supplying unpasteurised milk.	Provided by UK Food Standards Agency
Probability herd is positive	p_{Herd}	$\begin{cases} 0.318 \ \ if H < 50 \\ 0.600 \ if \ 50 \ \leq H \leq 100 \\ 0.781 \ if \ H > 100 \end{cases}$	McCaughey *et al.* (2010)
Probability animal is positive given herd is positive	p_{Within_herd}	$\begin{cases} 0.034 \ \ if H < 50 \\ 0.102 \ if \ 50 \ \leq H \leq 100 \\ 0.125 \ if \ H > 100 \end{cases}$	McCaughey *et al.* (2010)
Probability animal is lactating	$p_{Lactating}$	Pert (265, Uniform (300,305); 340)/365	ARC (2013).
Probability animal is shedding *C. burnetii* in milk given animal is lactating and infected	$p_{Shedding}$	22 of 72 infected cows (0.3055)	Guatteo *et al.* (2012)
Volume of milk (per animal per day)	V_i	Normal (25.6, 1.263) (litre)	Kingshay (2013)
Coxiella burnetii concentration in milk (Shedders)	C_{ml}	Guinea pig intraperitoneal ID_{50} per ml distributed as 0.5 x $10^{Normal\ (1.333,\ 1.0847)}$	Enright *et al.* (1957)
Cumulative milk consumption per person per day	$M_{Litre/Day}$	0.127 (litre per person per day)	Department of Health (2011)

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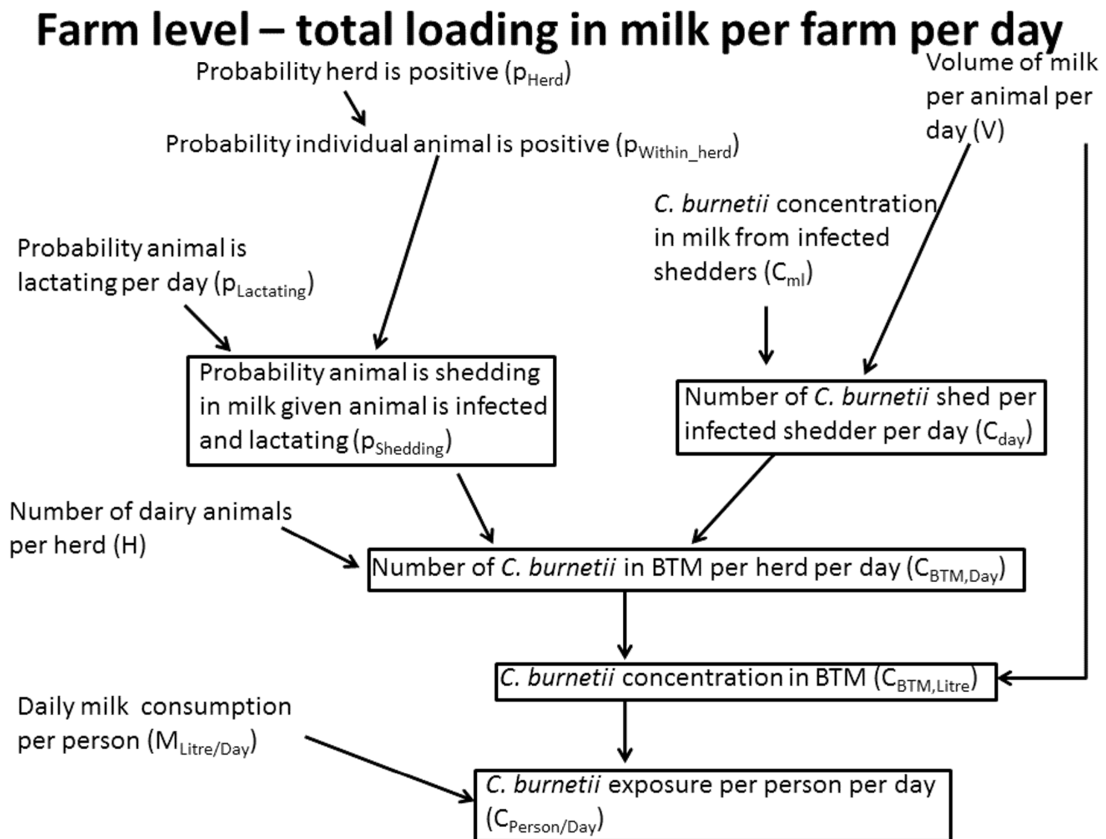

Figure 1: Schematic diagram for the probability of exposure and levels of *C. burnetii* per person per day through consumption of unpasteurised milk. The model outputs are in boxes.

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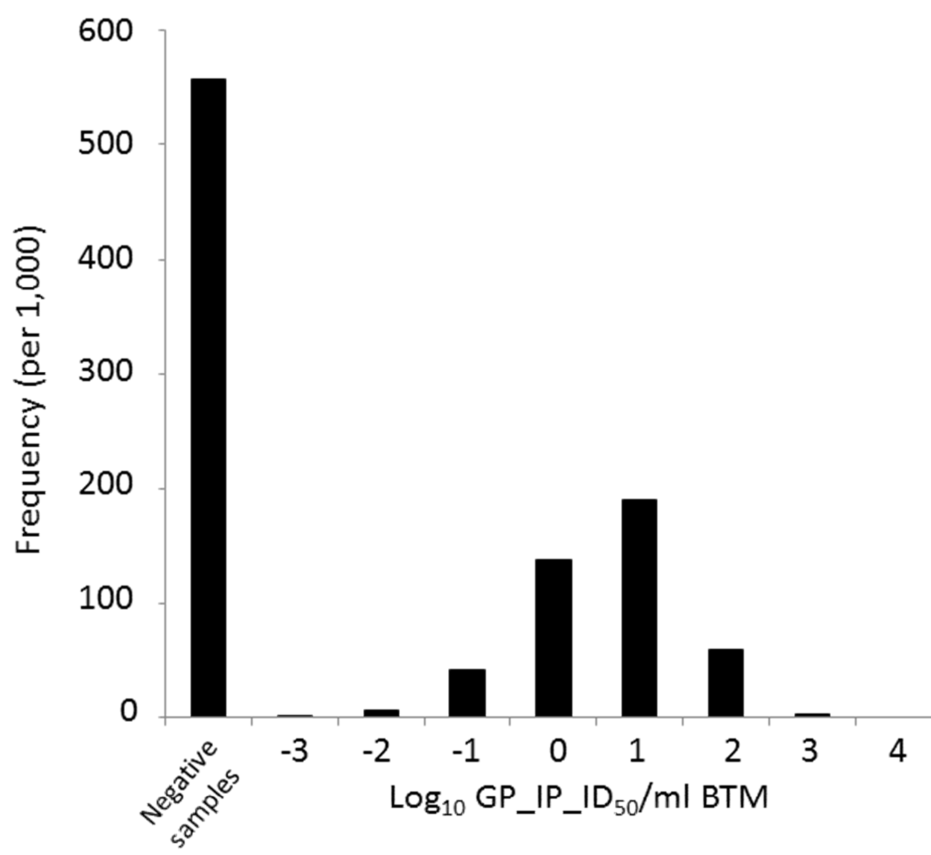

Figure 2: Simulated $GP_IP_ID_{50}$s of *Coxiella burnetii* per ml of unpasteurised BTM milk plotted on a log scale for comparison with qPCR data of Valergakis *et al.* (2012).

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