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

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Executive summary

Q fever is a widespread, zoonotic disease caused by the bacterium *Coxiella burnetii* which is endemic in livestock including cattle, sheep and goats in the United Kingdom (UK). The clinical manifestations of Q fever in humans are variable, ranging from asymptomatic to serious. It is known that viable *C. burnetii* can be shed in milk, although the link between infection and clinical disease in humans through consumption of unpasteurised milk and milk products is unclear.

The original aim of this work was to undertake a quantitative risk assessment for *C. burnetii* in unpasteurised milk and milk products from UK cattle, goats and sheep. However, in part due to the problems in culturing this pathogen, much of the data required for the risk assessment are missing. For example, while there are data from the 1950s on the number of GP_IP_ID_{50} units[1] 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. It was therefore agreed with the Food Standards Agency to develop a risk profile for *C. burnetii* through unpasteurised milk and milk products and, instead of developing a full risk assessment, to conduct an exposure assessment for consumption of unpasteurised cows' milk.

The main conclusion of the risk profile is that unpasteurised milk and milk products may contain viable *C. burnetii*. Therefore the risks of human infection from *C. burnetii* through consumption of unpasteurised milk and milk products are not negligible. However, current knowledge suggests a low risk to human health from ingestion through milk particularly when compared to aerosols from parturient products and livestock contact. This reflects not only the more efficient transmission via inhalation of contaminated aerosols in which inhalation of just a few organisms may be sufficient to initiate infection but also the much higher loadings in birth products compared to milk, potentially giving higher exposures across the population through aerosols. *C. burnetii* infectivity, however, persists in milk and milk products over long periods. Information gathered on the methods used to produce unpasteurised milk products such as cheese (hard and soft), butter and cream suggest that no manufacturing/process steps (other than pasteurisation) would result in a significant reduction in *C. burnetii* present in unpasteurised milk, although long maturation at low pHs may give some inactivation in hard cheese. 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.

Taking into account, the prevalence in UK cattle according to herd size and the probability of shedding and lactating, the mean level of *C. burnetii* was predicted to be 4,189 GP_IP_ID_{50} per litre of unpasteurised milk from the bulk tank with 2.5th and 97.5th percentiles of 0 and 26,848 GP_IP_ID_{50} per litre,

[1] Guinea Pig, IntraPeritoneal Infectious Dose 50%, i.e. dose when given to each of a group of guinea pigs through intraperitoneal challenge results in 50% being infected

respectively. Although seemingly high, these predictions are not inconsistent with recently published data from PCR studies on bulk milk tank samples taken in south-west England. The exposure assessment predicted that the probability of exposure to viable *C. burnetii* 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. However, based on an assumed low risk of infection from oral exposure as per the evidence collated in the risk profile, it is likely that these predicted daily oral exposures present a relatively low risk to public health.

CONTENTS

Glossary

Term	Description
BTM	Bulk tank milk
qPCR	Quantitative PCR – amplifies and simultaneously quantifies a target DNA sequence
PCR	Detects presence of specific DNA sequence
ELISA	Enzyme-linked Immunosorbent Assay – detects presence of antibodies to specific pathogen in blood serum
$GP\_IP\_ID_{50}$	Guinea Pig IntraPeritoneal Infectious Dose 50% - the dose which when given to each and every member of a group of guinea pigs through intraperitoneal challenge results in 50% being infected.
SCV	Small cell variant – more resistant morphotype of *C. burnetii*

1. INTRODUCTION

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*, which is present in livestock, including cattle, sheep and goats. 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. The clinical manifestations of Q fever in humans are variable. Acute Q fever in humans usually manifests as an asymptomatic or mild flu-like disease with spontaneous recovery. However, a small minority of patients present with more serious disease which can lead to serious complications and death. 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. Epidemiological evidence suggests that the main route of transmission to humans is via the inhalation of aerosols from the parturient (birth) products of infected mammals, including livestock. It is known that viable *C. burnetii* can be shed in milk, although the link between infection in dairy livestock and clinical disease in humans through consumption of unpasteurised milk and milk products is unclear (EFSA, 2010).

1.1 ***Scope and aims***

The original proposal to the Food Standards Agency (FSA) specified a full farm-to-consumption quantitative microbiological risk assessment to assess the risk to humans from the consumption of unpasteurised milk and milk products made from unpasteurised milk of United Kingdom (UK) cattle, sheep and goat origin. The risk assessment was to follow the main steps of the Codex risk assessment framework (CAC, 1999), which is most commonly used for food safety risk assessments. Using the Codex framework, a risk assessment is split into the following components; namely, Hazard Identification, Exposure Assessment, Hazard Characterisation and Risk Characterisation. However, during the Hazard Identification stage of the risk assessment it became clear that there were significant data gaps in the level of knowledge of *C. burnetii*. In particular there was little or no information on:-

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

Those data gaps in part reflect the difficulties in routine culture of *C. burnetii* and also the lack of data on the viability of the organisms when DNA is detected by PCR methods. It was therefore agreed by the FSA project team that an alternative approach was required as there were insufficient data for a full quantitative or qualitative risk assessment. Overall, the revised work

programme included four deliverables. These are set out in Appendices 1 to 4 of this project report and include:-

1. The hazard identification
2. The development of a risk profile for *C. burnetii* through consumption of unpasteurised milk and milk products;
3. The identification of the risk pathways to humans through consumption of unpasteurised milk and unpasteurised cheeses; and
4. The development of an exposure assessment model, together with a sensitivity analysis, for humans consuming unpasteurised cows' milk.

Of additional interest to the FSA was the risk to humans due to consumption of unpasteurised milk/milk products during an abortion storm at a goat farm. In the absence of sufficient data for goats, a scenario analysis was undertaken for cows' milk to represent an outbreak within a cattle herd.

2. HAZARD IDENTIFICATION

The hazard identification is provided in Appendix 1. The key points from the hazard identification are summarised here.

2.1 ***The organism***

C. burnetii is an obligate intracellular bacterium that relies exclusively on a eukaryotic cell for growth. Key characteristics in regard to risks through food and environmental routes 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).

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

2.2 ***Routes of infection***

There are a number of routes identified by epidemiological studies for transmission of *C. burnetii* to humans. The main routes of transmission are from livestock and companion mammals 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 of Q

fever arising from *C. burnetii* sources many years after release from an infected mammal.

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 unpasteurised milk was the most likely cause. The most recent of these was in Michigan (USA) in 2011 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 unpasteurised milk being the source of the outbreak, there is still uncertainty associated with this link. With the possible exception of an outbreak in France 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.

3. **RISK PROFILE**

The objective of the risk profile was to provide 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. The full risk profile is available in Appendix 2.

3.1 ***Shedding and prevalence in UK livestock***

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. Reported prevalences in bulk tank milk (BTM) samples from dairy cattle herds in England and Wales range from 22% (ELISA) to 69.7% (PCR). 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 *C. burnetii* in milk and in the case of some cattle, duration of shedding may be for more than one year. 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 dairy cattle in south-west England. However, the problem with PCR is that it gives no information on the viability. 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.

3.2 ***Presence and viability of C. burnetii in milk and milk products***

C. burnetii is clearly viable to some degree in unpasteurised milk because experiments conducted in the 1940s/50s showed that naturally infected cows' milk can infect guinea pigs and mice albeit through intraperitoneal challenge. Thus, Enright et al. (1957) used a guinea pig bioassay approach to measure *C. burnetii* in unpasteurised cows' milk. The great advantage of guinea pig bioassay over PCR data is that it determines viable pathogen. Levels of *C. burnetii* are thus expressed in units of "guinea pig intraperitoneal infectious

dose 50%" or GP_IP_ID_{50} which 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) reported that milk from 18 of 137 individual cows in a naturally-infected dairy herd contained viable *C. burnetii*. The numbers of *C. burnetii* GP_IP_ID_{50} per 2 ml of milk from those 18 shedding cattle are plotted in Figure 1 and a lognormal distribution was fitted to these data for subsequent use in the exposure assessment. The mean number of *C. burnetii* GP_IP_ID_{50} per ml of milk is 98.8.

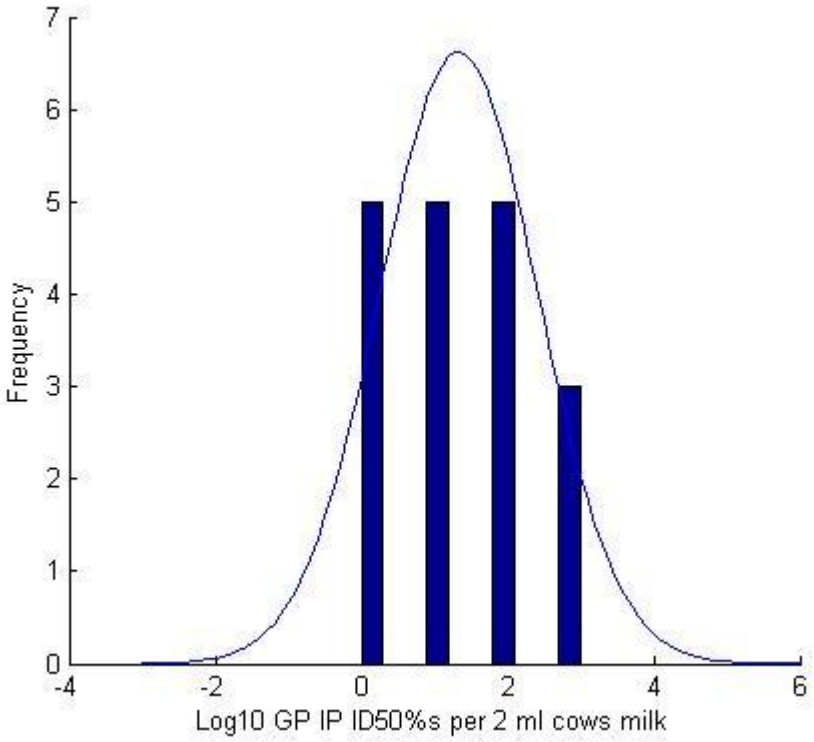

Figure 1: Fitted normal distribution for log_{10}-transformed numbers of GP_IP_ID_{50}s per 2 ml of milk from 18 naturally infected cows which are shedding *C. burnetii* in milk, i.e. positives only (data from Enright et al 1957).

3.3 ***Comparison of viability in unpasteurised milk and unpasteurized cheese at point of purchase***

Although *C. burnetii* is inactivated by pasteurization, it is unlikely that any of the processes used for unpasteurised cheese, cream or butter production would significantly inactivate *C. burnetii*. Some pathogen may be removed with the whey during cheese production, although no data are available to assess whether this is significant. More recently, a number of PCR studies have detected the presence of *C. burnetii* DNA in unpasteurised (and pasteurised[2]) milk and unpasteurised cheese at point of purchase. From viability studies in mice, the few available results suggest that *C. burnetii* may be viable in unpasteurised milk but not in cheeses made from unpasteurised

[2] Pasteurisation is effective in inactivating *C. burnetii* (Enright et al. 1957) even though DNA may still be detectable.

milk; however it is noted that there are very few studies which have investigated the viability in cheese. Thus, while more extensive sampling of unpasteurised cheeses could detect viable *C. burnetii*, it is also possible that the combination of time/process conditions (e.g. lower pH and longer maturation times) in cheese-making is not conducive to survival of *C. burnetii*. Indeed Eldin et al. (2013) "believe that *C. burnetii* does not survive in cheese (and yogurt)". This is consistent with the epidemiological findings that in the developed world there is some evidence for human Q fever outbreaks where consumption of unpasteurised milk was the most likely cause while there is little evidence linking Q fever to consumption of unpasteurised cheeses, which suggests the cases through cheese are sporadic and, compared to unpasteurised milk, are lower in frequency.

3.4 ***Dose-response***

C. burnetii is highly infectious through inhalation with some authors estimating the risk of infection from a single bacterium to be as high as 90%. While human feeding studies have been reported (Fonseca et al. 1949), the doses administered were not specified. Assessing the risk of transmission through milk and milk products requires data on the infectivity through the oral route, as opposed to the inhalation route for transmission through aerosols from mammal birth products or livestock. A key finding from the work on the risk profile was that infectivity may be lower through the oral route than the inhalation route, probably reflecting the smaller numbers of target cells (macrophages) in the gastrointestinal tract compared to lung tissue. Indeed, Cerf and Condron (2006) even challenge the designation of *C. burnetii* as a foodborne pathogen. 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.

3.5 ***Risks through milk and milk products compared to other routes***

As part of the risk profile, other routes of infection in addition to milk and milk products were briefly considered to allow comparison and thus provide an indication of the relative importance of the milk/milk product route in the overall epidemiology of *C. burnetii*. There is strong epidemiological evidence for aerosol transmission including windborne transmission of Q fever from livestock farms, slaughter houses and meat processing plants to people in nearby towns. Contact with farm animals has been found to be a major risk factor. In contrast, the epidemiological evidence for transmission through milk is much weaker. This is consistent with the *C. burnetii* bacterium being less infectious through milk products compared to aerosolised bacteria from livestock births or abortions. In addition, the exposures to humans may be lower through consumption of unpasteurised milk products than through aerosols. Huge numbers of bacteria are produced during abortion caused by *C. burnetii* and via livestock birth products (10^9 GP_IP_ID_{50}s per gram of placenta) compared to the mean of 98.8 GP_IP_ID_{50}s per ml of unpasteurised milk from shedding cows (Figure 1). 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, although it is not clear how many genome equivalents comprise a $GP\_IP\_ID_{50}$[3]. Furthermore there is some evidence developed in this work and discussed in Appendix 4 that *C. burnetii* in placental tissue may be more infectious (per bacterium) than in milk.

3.6 ***Differences in risk profile for cattle, sheep and goats***

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. Indeed, for goats, milk is the main route of shedding with 31 – 38% shedding in milk if infected (Rousset et al. 2009). In terms of consumption patterns the use of cows' and goats' milk seems to be more common than for sheeps' milk. Indeed, unpasteurised cheese and yoghurt are normally made from cows' or goats' milk.

3.7 ***Overall conclusions of risk profile***

The overall conclusion of the risk profile was that the risks of *C. burnetii* infection to humans 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. 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. 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 and low pH. 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. Future studies could involve using qPCR to estimates levels of *C. burnetii* DNA in the whey and curds.

Due to uncertainty in much of the data, a risk assessment for infection through milk and milk products cannot be undertaken at present. These knowledge gaps in levels of viable pathogen in livestock herds and in milks, particularly from goats and sheep, survival of *C. burnetii* in milk products and dose-response are compounded by the experimental obstacles in culturing *C. burnetii* associated with intracellular obligatism (Omsland and Heinsen 2011). PCR, although advantageous in terms of rapid identification has had a clear disadvantage on obtaining data useful for risk assessment in that, although, the number of genomic equivalents can be quantified, their viability is not known. In this respect, the guinea pig/mice infection assays undertaken

[3]The $GP\_IP\_ID_{50}$ from the placenta may comprise just one *C. burnetii* organism. Thus Kersh et al. (2013) recorded 1.5 to 2.5 x 10^8 genome equivalents per gram of placenta from goats which agreed well with the 5.0 x 10^8 average $GP\_IP\_ID_{50}$ per gram from ovine placental tissue (Welsh et al. 1951). Hansen et al. (2011) reported 10^9 *icd* gene copies (single copy per bacterium) per ml of eluate from cattle cotyledons in parturient cattle.

before the advent of PCR offer much greater value for risk assessment. A key recommendation of this work is that methods be developed for detecting and enumerating viable *C. burnetii*.

4. RISK PATHWAYS FOR HUMANS THROUGH CONSUMPTION OF UNPASTEURISED MILK AND MILK PRODUCTS

The pathways and data requirements for occurrence of *C. burnetii* in bulk tank milk at the herd level and for infection of humans due to consumption of unpasteurised milk and unpasteurised cheese are set out in Appendix 3. This includes an assessment of the availability of data for each of the steps. The pathways are generic and can be parameterised for cow's or goats' milk/cheese.

5. EXPOSURE ASSESSMENT FOR CONSUMPTION OF UNPASTEURISED COWS' MILK

Taking into account the available data and the potentially greater risks through milk compare to cheese, the risk question to be addressed is:

What is the exposure of C. burnetii to a consumer through the cumulative consumption of unpasteurised cows' milk over the period of a day?

To address this, the quantitative estimation of exposure was broken down into two outputs, namely:-
1. The probability of exposure through the cumulative daily consumption of unpasteurised milk; and
2. The level of exposure, given exposure has occurred, to a person through consumption of unpasteurised milk over the period of a day.

As exposure pathway developed to address these questions is shown in Figure 2. The pathway identifies the data requirements for the parameterisation of the exposure model. Full techincal details of the exposure model are provided in Appendix 4.

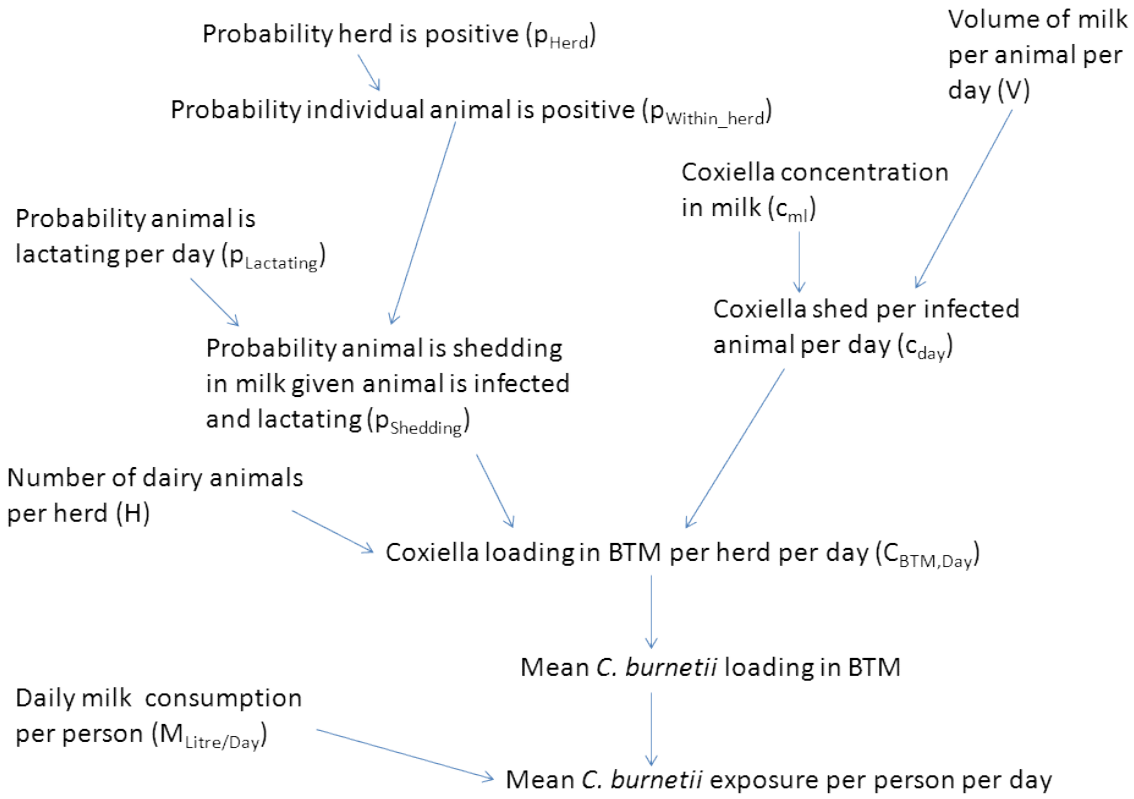

Figure 2: Schematic diagram for the probability of exposure and levels of *C. burnetii* per person per day through consumption of unpasteurised milk

*5.1 **Summary of available data***

***5.1.1** Data used for estimating levels of C. burnetii in bulk tank milk (per herd) for cattle in the baseline model*

A summary of the model parameters used for the baseline model is given in Table 1. It should be noted that the herd sizes used represented those for the 81 herds in England and Wales producing unpasteurised milk as shown in Figure 3. The between herd and within herd prevalences used reflect those for Northern Ireland (McCaughey et al. 2010) and are broken down according to herd size. For the baseline model it is assumed that a cow that is infected and shedding does so for every day of the year. This is a worst case assumption but reflects the fact that some naturally infected cattle can shed for at least a year (Enright et al 1957).

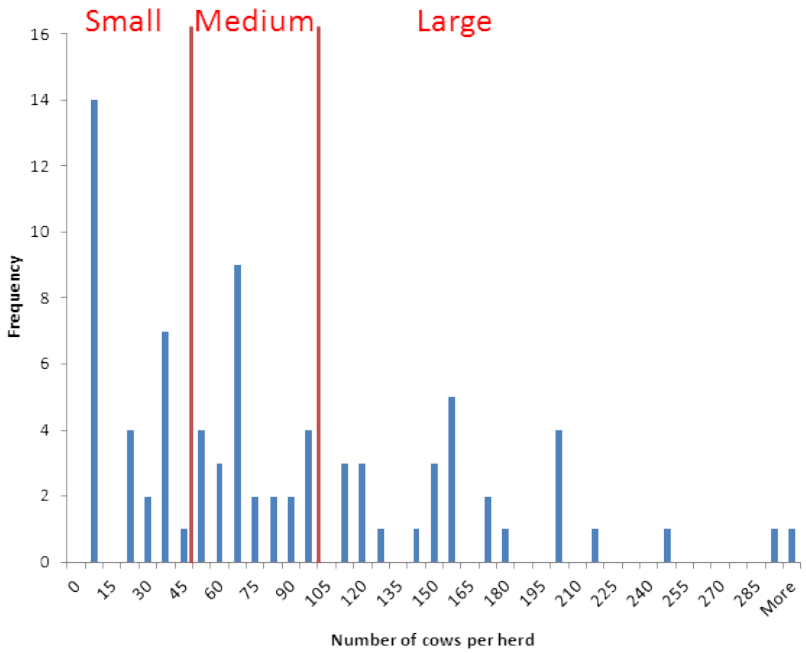

Figure 3: Histogram for number of dairy cattle per herd for the 81 herds supplying unpasteurised milk for human consumption in England and Wales. Data from FSA.

The numbers of *C. burnetii* GP_IP_ID_{50} per 2 ml of milk from shedding cattle as used in the exposure assessment are those presented in Figure 1, the logs of which are described by a normal distribution (Table 1).

5.1.2 *Data for daily consumption of milk*

The mean consumption for raw milk used in the baseline risk assessment is 0.127 kg/person/day based on data for whole milk (presumably pasteurised) consumption in the 19-64 year old age group (Department of Health 2011). Using this value in the exposure assessment represents the cumulative daily mean exposure to *C. burnetii* through consumption of milk in units of per person per day. This is worst case in the sense that it assumes all milk consumed per day by an individual is raw and that none of this raw milk is subjected to any heat treatment or added to a hot drink. It was assumed that no decay of the *C. burnetii* occurred in the time period between milking and consumption.

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 values or distribution used in @RISK	Reference
BASELINE MODEL			
Number of dairy cows per herd	H	Used empirical data for 81 cattle herds in England and Wales supplying unpasteurised milk (Figure 3).	Provided by FSA
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 $0.5 \times 10^{Normal\ (1.333,\ 1.0847)}$	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
OUTBREAK SCENARIO			
Probability animal is positive given herd is positive	P_{Within_herd}	0.389 for all herd sizes	Rodolakis et al. (2007)
Probability animal is shedding *C. burnetii* in milk given animal is lactating and infected	$P_{Shedding}$	0.92	Rodolakis et al. (2007)

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

The quantitative model is 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. Each iteration of the model represents the milk produced from a single herd on a given day. In this respect each simulated concentration represents the mean for the levels of *C. burnetii* in the BTM for a day. This is consistent with the fact that milk in the bulk tank is stirred, and in the case of unpasteurised

milk is not mixed with milk from other cattle herds. Taking into account the herd prevalence, the within herd prevalence, the probability of shedding (Table 1) and using the data for levels of infectivity in milk from infected cows which are shedding (Figure 1) the simulated mean level of *C. burnetii* is 4,189 GP_IP_ID_{50} per litre of unpasteurised milk from the bulk tank with a 97.5^{th} percentile in the simulation of 26,848 GP_IP_ID_{50} per litre. This represents the mean for the 81 unpasteurised milk-producing herds in England and Wales over a total of 500,000 simulated days.

5.2.1 *Validation of predicted levels of infectivity in unpasteurised milk against published PCR data*

These seemingly high values for the mean and 97.5^{th} percentile levels in BTM reflect the values of up to 1,000 GP_IP_ID_{50}s per 2 ml of unpasteurised milk to which the log-Normal distribution, used in the simulation here, was fitted (Figure 1). The distribution for the number of *C. burnetii* GP_IP_ID_{50} per ml of BTM milk as simulated is presented in Figure 4. The GP_IP_ID_{50}s per ml are converted to logarithms to enable direct comparison with the distribution from Valergakis et al. (2012) of the qPCR units per ml of milk in Figure 5. The two distributions are similar *in shape* with each having two peaks. The zero 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 (Figure 5). Thus initial consideration would suggest that the model is underestimating 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 some of 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;
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}. Indeed comparison of quantitative PCR results of Guatteo et al. (2007) for *C. burnetii* in dairy milk with the GP_IP_ID_{50} recorded in milk by Enright et al. (1957) (Figure 1) suggest there could be between 2 and 112 *C. burnetii* organisms per GP_IP_ID_{50} in milk.

It is concluded, therefore, that the predictions of GP_IP_ID_{50} in BTM (Figure 4) are not inconsistent with the published PCR data for BTM (Figure 5). Thus if each GP_IP_ID_{50} comprised 50 bacteria each with 20 copies of the PCR target sequence, 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 4) and observed number of PCR copies/ml (Figure 5).

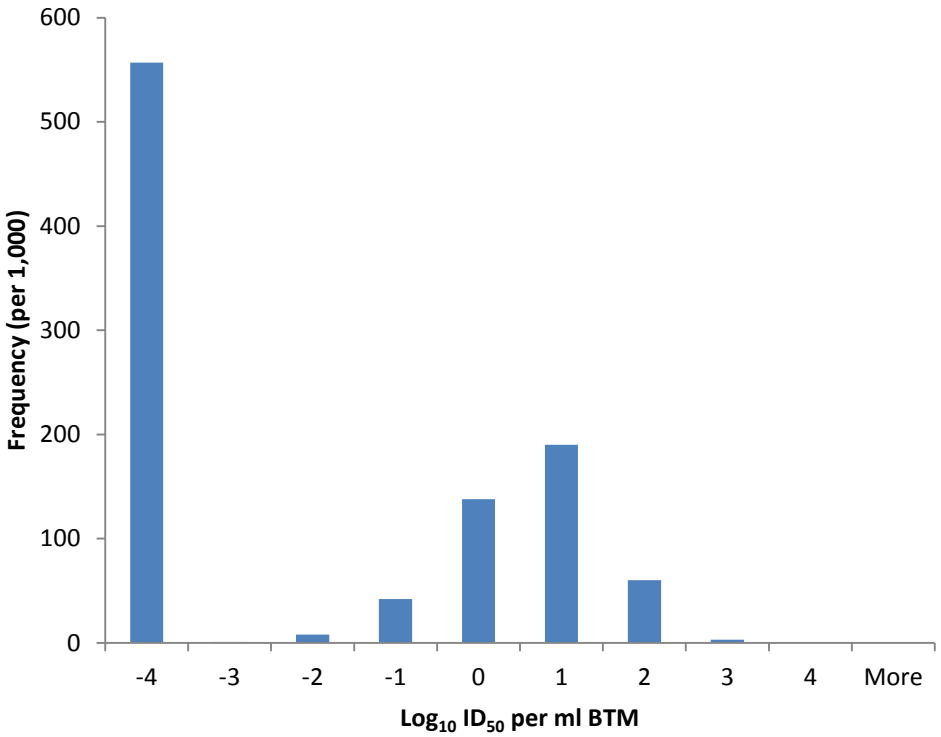

Figure 4: Simulated GP_IP_ID_{50}s of *Coxiella burnetii* per ml of unpasteurised BTM milk plotted on a log scale for comparison with Figure 5. 1,000 samples simulated under baseline conditions for purpose of graphical presentation; zero per ml represented as -4.

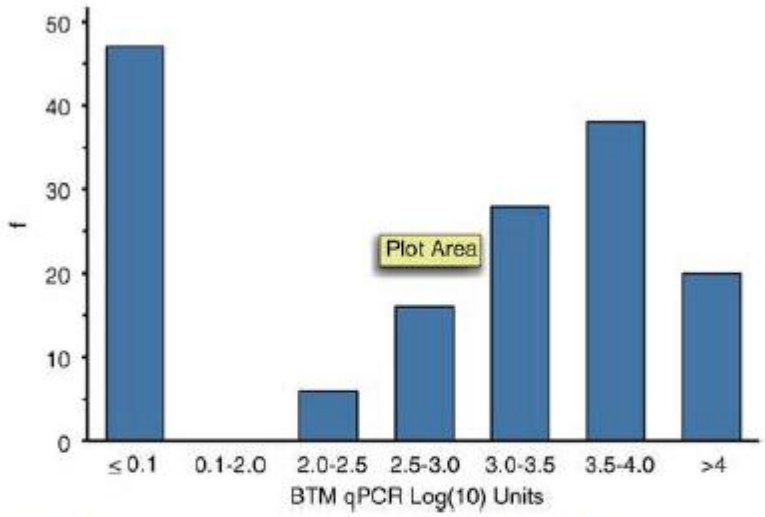

FIG 1: Histogram showing frequency of real-time PCR results for *Coxiella burnetii* in dairy herds (n=155) in south-west England, expressed as $\log_{10}$ of the number of *C burnetii* per ml of bulk tank milk

Figure 5: Quantitative PCR results for *C. burnetii* DNA in BTM (Valergakis et al. 2012)

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

5.3.1 *Baseline model*

The baseline exposure assessment predicted that the probability of exposure to viable *C. burnetii* 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 exposures estimated by the baseline simulation 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 assumes that all *C. burnetii* DNA in milk from an infected cow does indeed represent viable *C. burnetii*.

Therefore the baseline results can be viewed as a worst case scenario.

5.3.2 *"Outbreak" scenario*

There is no statutory requirement to submit abortion material for Q fever surveillance in the UK. Reporting is voluntary and cases may go unreported. Although *C. burnetii* abortion storms have not been reported in UK cattle, an "outbreak scenario" was simulated assuming higher within herd prevalences together with a higher proportion of infected cattle shedding in milk. The changes to the model are summarised in Table 1.

In the "outbreak scenario", the probability of exposure through consumption of unpasteurised milk increased from 0.4203 to 0.5453 per person per day. This relatively small increase reflects the fact that the between herd prevalence was not changed in the outbreak simulation and thus a proportion of herds are still negative and hence their milk is negative too. In contrast the mean exposure per positive serving increased nine-fold from 1,266 to 11,612 $GP\_IP\_ID_{50}$ per person per day (with 2.5^{th} and 97.5^{th} percentiles of 342 and 54,605 $GP\_IP\_ID_{50}$ per person per day, respectively).

5.3.3 Risk to public health

The available PCR data (Figure 5) and 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 guinea pig intraperitoneal infectious dose 50% (GP_IP_ID_{50}) units into human oral ID_{50}s (because of lack of human dose-response data), it is likely that each one presents a low risk to humans through the oral route. Fonseca et al. (1949) demonstrated high infection rates by *C. burnetii* in humans through intradermal challenge but low risks through oral challenge (although it is not known if the challenge doses were the same) thus adding weight to this hypothesis. 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)). A key point for the oral route is the lower numbers of target macrophages in the gastrointestinal tract compared to the lungs. Thus the lung tissue with a high number of alveolar macrophages is a prime environment for initial infection by *C. burnetii* and is the most common route of infection (Mike Minnick, Montana University, pers comm.). Furthermore, 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).

5.4 ***Sensitivity analysis***

A major source of uncertainty in this model is the duration of shedding in milk by infected cattle. The baseline model assumed continuous shedding for every day of the year that they are lactating. In a "short duration shedding" scenario in which infected (and shedding) cattle only shed for 1 month of the year compared to all year, not only was the probability of exposure decreased four-fold (from 0.4203 to 0.1048 per person per day) but also the mean level of exposure for positive daily exposures was decreased three-fold relative to the baseline model.

The baseline risk assessment is based on data for levels of infectivity in unpasteurised milk from naturally infected cows (Figure 1). Including a maximum of 20,000 GP_IP_ID_{50} per 2 ml of unpasteurised milk from an experimentally infected cow in the fitting process to include a high shedder, little affected the probability of exposure per person per day but increased the mean level of exposure by about four-fold in those who were exposed.

Assuming all herds were positive had relatively little effect on the mean daily exposure per person for positive exposures, but did increase the probability of exposure from 0.4203 to 0.6271 per person per day. Doubling the amount of unpasteurised milk consumed daily per person had relatively little effect.

Assuming all cows within a positive herd are infected increased the probability of exposure from 0.4203 to 0.5424 per person per day and increased the mean level of exposure (in those who were exposed) by six-fold.

In terms of the sensitivity, the factors which had the biggest impact on total exposure through consumption of raw milk were the proportion of infected cattle shedding, the within herd prevalence and the duration of shedding.

6. **CONCLUSIONS**

The main conclusions of the risk profile and exposure assessment conducted here are:-

1. *C. burnetii* is endemic in cattle, sheep and goats in the UK.
2. *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 in part accounts for the significant data gaps/deficiencies across the farm-to-consumption exposure pathway and hazard characterisation. In this respect, a recommendation of this work is that laboratory methods are developed for detecting and enumerating viable *C. burnetii*.
3. *C. burnetii* has spore-like environmental stability due to a resistant morphotype which probably exists in milk and accounts for the survival of infectivity in milk and milk products over long periods. Information gathered on the methods used to produce unpasteurised milk products such as cheese (hard and soft), butter and cream suggest that no manufacturing/process steps (other than pasteurisation) would result in a significant reduction in *C. burnetii* present in unpasteurised milk, although long maturation at low pHs may give some inactivation in hard cheese. 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.
4. 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.
5. There is epidemiological evidence that a small proportion of cases of Q fever in the developed world may arise from exposure to *C. burnetii* through consumption of unpasteurised milk, and indeed viable *C. burnetii* have been detected in unpasteurised milk.
6. 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 are not negligible but they are lower in comparison to transmission via inhalation of aerosols from parturient products and livestock contact.
7. 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 mammals and/or direct contact with infected livestock; a belief shared by other researchers in this area. This is attributable to the relatively lower loadings of *C. burnetii* in milk

compared to placenta and also to the lower assumed infectivity of this pathogen though the oral route compared to the inhalation route. Thus, the lower infectivity of *C. burnetii* through the oral route compared to the inhalation route probably reflects the lower numbers of target macrophages in the gastrointestinal tract compared to the lungs (Mike Minnick, Montana University, pers comm.). There is also some tentative evidence to suggest that the pathogen is less infectious in milk than in placentas (per DNA copy), although this needs further substantiation.

8. *C. burnetii* DNA is detectable using PCR in unpasteurised (and also pasteurised) milk in the UK. Cows and goats shed the DNA mainly in milk while ewes shed mostly in faeces and vaginal mucus. It is clear that some of that DNA in milk represents viable pathogen.
9. Some data are available for levels of viable *C. burnetii* in naturally infected cows' milk, but not for goats' or sheep's milk. For milk from infected cows which were shedding *C. burnetii*, the mean level of infectivity was 98 guinea pig intraperitoneal infectious dose 50% units ($GP\_IP\_ID_{50}$) per ml of milk.
10. Taking into account the prevalence in UK cattle according to herd size and the probability of shedding and lactating, the mean level of *C. burnetii* was predicted to be 4,189 $GP\_IP\_ID_{50}$ per litre of unpasteurised cows' milk from the bulk tank (2.5^{th} and 97.5^{th} percentiles of 0 and 26,848 $GP\_IP\_ID_{50}$ per litre of unpasteurised milk, respectively). Although seemingly high, these predictions are not inconsistent with published data from PCR studies on bulk milk tank samples taken in south-west England.
11. Cumulative consumption of unpasteurised milk in the UK is assumed to be 0.127 litres per person per day on the basis of data for whole milk that had presumably been pasteurised. There is little information for the consumption patterns of unpasteurised milk and milk products broken down according cattle, goat or sheep origin.
12. Exposure assessments conducted here predict that the probability of exposure to viable *C. burnetii* through consumption of unpasteurised milk in the UK is 0.4203 per person per day and that the exposure to those who are exposed will be relatively high (mean 1,266 $GP\_IP\_ID_{50}$ per person day with 2.5^{th} and 97.5^{th} percentiles of 2 and 7,524 $GP\_IP\_ID_{50}$ per person per day, respectively). Although there are no quantitative dose-response data for humans through the oral route it is likely that these predicted daily exposures through consumption of unpasteurised milk present a relatively low risk to public health.
13. The prediction that almost 60% of daily exposures through unpasteurised milk are negative reflects the fact that many herds are not infected in the UK.
14. In terms of the sensitivity, the factors which had the biggest impact on total exposure through consumption of raw milk were the within herd prevalence and the duration of shedding.

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