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

Milestone Report M03/02 – M03/04: Human exposure: *Coxiella burnetii* in unpasteurised milk

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Executive summary

Q fever is a zoonotic widespread disease caused by the bacterium *Coxiella burnetii* which is endemic in cattle, sheep and goats in the UK. There is strong epidemiological evidence that a small proportion of cases in the developed world may arise from exposure through consumption of unpasteurised raw milk, and indeed viable bacteria have been detected in raw milk. The infectivity of *C. burnetii* through the oral route is likely to be much lower than through inhalation and currently there are no dose-response data for humans through the oral route. Levels of *C. burnetii* in raw milk from naturally infected cows were published in the 1950s and quantify the pathogen in terms of guinea pig intraperitoneal infectious dose 50% units ($GP\_IP\_ID_{50}$) per volume of milk. Taking into account other data including prevalence in UK cattle according to herd size and probability of shedding, an assessment is presented here to model the exposures to *C. burnetii* for humans through consumption of 0.127 litres of raw milk per person day. This volume of raw milk is based on published Department of Health data for the total cumulative daily consumption of whole milk (presumably pasteurised) per person in the 19 to 64 year age group in the UK (and assumes not only that an individual who drinks raw milk drinks the same volume of milk as someone who drinks pasteurised milk but also that the raw milk is not heat treated or consumed with hot drinks). The results of the simulation predict that almost 60% of daily exposures are negative reflecting the fact that many herds are not infected in the UK. However, those 40% of daily exposures which were positive for *C. burnetii* gave an average exposure of 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 day). Although seemingly high, these predictions are not inconsistent with published data from PCR studies on bulk milk tank samples taken in south-west England. It should be noted that a $GP\_IP\_ID_{50}$ unit may represent a relatively low risk through the oral route, although there are no data to relate $GP\_IP\_ID_{50}$ units to risk of oral infection in humans. In terms of the sensitivity for the scenarios selected, the model parameters which had the biggest impact on total exposure through consumption of raw milk were the within herd prevalence and the duration of shedding.

CONTENTS

1. **INTRODUCTION**

Q fever is a zoonotic widespread disease caused by the bacterium *Coxiella burnetii* which is present in cattle, sheep and goats. It is an emerging disease that can cause considerable morbidity and serious long-term complications in humans. Despite the uncertainties and data gaps/deficiencies there is some strong epidemiological evidence, from the developed world, that cases of Q fever have occurred where consumption of raw milk was the most likely cause. The number of registered producers who provide raw cows' milk for direct human consumption in England and Wales has fallen from around 570 in 1997 to around 100 in 2010 (FSA 2012, para 4.12) with 81 dairy cow herds in England and Wales supplying raw milk in 2012 (FSA data). The total number of cows on those 81 farms in 2012 was 7,011 animals.

The risk question agreed in the proposal was:

What is the probability of human infection with Q fever due to the consumption of a serving of unpasteurised milk or milk products?

However, as discussed in the risk profile (Milestone Report to FSA M02/01), critical data on the infectivity of *C. burnetii* in milk through oral challenge in humans are lacking. Indeed there are no quantitative dose-response data for *C. burnetii* through the oral route and for this reason the risk question was revised to address the magnitude of exposure to *C. burnetii* through consumption of milk and milk products.

1.1 ***Scope of risk assessment with regard to milk and milk products***

The quantities of the different (presumably pasteurised) milk products consumed in the UK are set out in Table 1 according to data from the Department of Health (2011). Raw milk is the least processed of the milk products shown in Table 1 and is generally consumed in the shortest time period (after milking from the animal) giving the least time for any inactivation to occur.

Although the productions of other milk products such as cheese, yoghurt, butter and ice cream involve processing steps, there are no quantitative data on survival of the pathogen during those processing steps. The amount of ice cream produced in the UK from raw milk is very small (Specialist Cheesemakers' Association, pers comm.). In the risk profile, no steps were identified that presented major barriers to *C. burnetii* in the production of cheese, yoghurt, butter or ice cream from raw milk. As discussed in the risk profile, there is some evidence that the low pH environment in some cheeses may promote inactivation of *C. burnetii* particularly over long storage periods, suggesting that the risks of exposure to viable organisms through some cheeses are lower than for milk. As noted in the risk profile, a major uncertainty is what happens to *C. burnetii* during the separation of whey from the curds during cheese-making.

Table 1: Mean quantities of milk and milk products (g/person/day) consumed* in UK according to age group (Department of Health 2011)

	1.5 – 3 yr	4 – 10 yr	11 – 18 yr	19-64 yr	65+ yr
Whole milk†	297	199	164	127	224
Semi-skimmed milk	185	176	138	128	169
1% fat	0	107	210	143	291
Skimmed milk	290	139	72	119	152
Other milk and cream	196	72	85	55	51
Cheese	12	16	18	25	21
Ice cream	21	28	33	29	30
Butter	5	8	9	9	13

*male and female consumers only, i.e. the lower figures based on both consumers and non-consumers are not presented here. Thus including non-consumers would lower the mean quantity/person/day because their contribution is zero.

†It is assumed for the risk assessment that those persons drinking unpasteurised milk would drink whole milk rather than semi-skimmed or skimmed because delivery companies in England do not appear to sell semi-skimmed or skimmed milks[1].

This lack of data together with the potentially greater exposures through milk resulted in a reconsideration of the risk question with regard to the scope of milk products. Moreover, quantitative data from the 1950s are available for levels of viable *C. burnetii* in milk of naturally infected cows thus supporting the case for an exposure assessment for raw milk in preference to other milk products. The modified risk question addressed here therefore is:

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

Here the quantitative estimation of exposure is considered. This may be broken down into two outputs, namely:-

[1] www.hookandson.co.uk and www.johnsjerseys,co.uk)

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

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

1.2 ***Aims***

The aims of this report are:-

- To quantify the exposure to *C. burnetii* through consumption of raw cows' milk produced by raw milk dairy herds in England and Wales in terms of the probability of exposure per day and the level of exposure;
- To compare the exposure under the endemic baseline scenario with a hypothetical outbreak scenario; and
- To test the sensitivity of the predicted exposures to changes in the magnitude of the input parameters.

2. MODEL OVERVIEW AND PATHWAY

Figure 1 shows the steps in the estimation of the probability and level of exposure (per person) from consumption of raw milk over the period of one day.

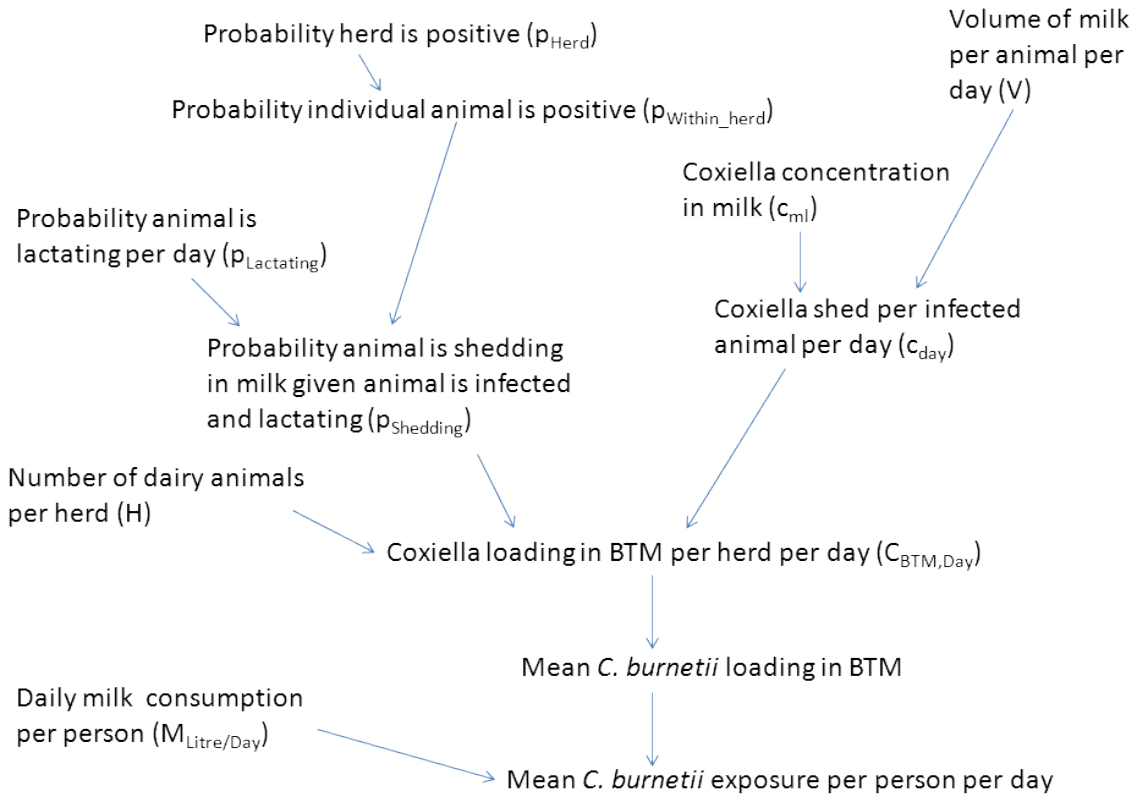

Figure 1: Schematic diagram for the probability of exposure and levels of *C. burnetii* per person per day through consumption of raw milk

While raw milk for human consumption, distribution and retail may be bottled in dairies in the USA (Loftis et al. 2010; Signs et al. 2012), legislation requires that raw cows' milk for sale in England and Wales is bottled on the farm premises for sale to the end consumer (either directly or through a distributor). Therefore no mixing of the milk between farms, as would be the case for pasteurised milk, is considered in the model. Furthermore, due to the lack of data for the survival/decay of *C. burnetii* it is assumed that there is no inactivation of any *C. burnetii* present in the milk during the time between milking and consumption. The output of the model is the *C. burnetii* exposure per person per day ($Raw\_Milk\_Exposure_{person/day}$) from which, both the

1. The probability of exposure; and

2. The level of exposure, given exposure has occurred;

are obtained. The quantitative model is implemented in the @RISK software package and incorporates variation associated with herds and individual animals in relation to infection, lactation and the levels of *C. burnetii* in milk.

It should be noted that each iteration of the model represents the milk produced from a single herd on a given day. Specifically each iteration of the model represents a herd of size H which may or may not be infected. Individual animals within the herd are classified as infected or not, lactating or not and for those that are infected and lactating, the volume of milk and the level of *C. burnetti* in that milk is simulated. The

classification of the herd and animals is undertaken using Bernoulli random variables, *Herd_Infected*, *Animal_Infected*, *Animal_Lactating* and *Infected_Animal_Shedding*". Randomly assigning a value of "1" indicates a positive status while a "0" indicates a negative status. Thus, whether or not the herd is infected is given by:

$$Herd\_Infected\_ = Binomial(1, p_{Herd})$$

where p_{Herd} is the proportion of infected herds. If the herd is infected then the infection status (Infected vs Not Infected) of each animal in the herd is given by:

$$Animal\_Infected\_ = Binomial(1, p_{Within\_Herd})$$

where p_{Within_Herd} is the proportion of cows infected in a positive herd. If the simulated herd status is negative, then the infection status of each animal is set to "Not Infected".

The lactation status of each cow in the herd is given by:

$$Animal\_Lactating = Binomial(1, p_{Lactating})$$

where $p_{Lactating}$ is the probability a given cow is producing milk on a given day. Given an animal is infected and lactating, the *C. burnetii* shedding status in milk is given by:

Equation 1 $$\boldsymbol{Infected\_Animal\_Shedding = Binomial(1, p_{Shedding})}$$

where $p_{Shedding}$ is the proportion of infected cows which shed *C. burnetii* in milk (given they are infected and lactating).

Whether or not a given animal in a herd is producing milk contaminated with *C. burnetii* on a given day is thus given by the random variable, *Positive_Milk*:-

$$Positive\_Milk = Herd\_Infected \times Animal\_Infected \times Animal\_Lactating \times Infected\_Animal\_Shedding$$

Given *Positive_Milk* = 1, the total number of *C. burnetii* shed per animal per day in milk, *N_Cburnetii*, is calculated as:

$$N\_Cburnetii = C_{ml} \times 1{,}000 \times V$$

where C_{ml} is the number of *C. burnetii* per ml of milk (from shedding cows) and V_i is the volume of milk yielded per animal *i* per day in litres. The mean concentration of *C. burnetii* per litre of milk from the herd on a given day is given by:-

Equation 2 $$\boldsymbol{Mean\_Raw\_Milk_{C.burnetii/litre}} = \frac{\sum_{i=1}^{H} N\_Cburnetii_i}{\sum_{i=1}^{H} V_i}$$

where *i* is each cow in the herd of size H. As mentioned above, raw milk cannot be mixed between farms if the product is to be sold as unpasteurised. The calculation in Equation 2 naturally estimates the mean number of *C. burnetii* per litre of milk by

summing the numbers of *C. burnetii* shed in milk by each and every cow in the herd per day and dividing by the total volume of milk produced by the herd per day. By doing this, information on the spatial distribution of *C. burnetii* in the milk is lost. Of course if the milk from the H cows in a herd is collected in a single tank (BTM) there will be an opportunity for some mixing of the contributions from the different cows. Indeed, although the milk is not homogenized there is a stirrer in the bulk tank (R. Mearns, AHVLA, pers comm). However, it should be noted that Equation 2 is assuming the bacteria are homogeneously distributed within the milk produced by a given herd on a given day. In the simulation, a value of $Mean\_Raw\_Milk_{Cburnetii/litre}$ is calculated for each of 10,000 iterations to give a distribution of 10,000 values. As mentioned above, each iteration of the model represents the milk produced from a single herd on a given day. In the simulation there are therefore 10,000 simulated herd-days. As discussed below (Section 3.1.1), herds of different sizes are simulated according to the FSA data provided on the number of cows per herd for 81 herds which supply raw milk for human consumption in England and Wales.

The mean exposure per person per day is calculated as the product of $Mean\_Raw\_Milk_{Cburnetii/litre}$ and the mean cumulative volume of milk consumed per person per day ($M_{Litre/Day}$), i.e.

$$Mean\_Raw\_Milk\_Exposure_{person/day} = Mean\_Raw\_Milk_{Cburnetii/litre} \times M_{Litre/Day}$$

The actual exposure is assumed to follow a Poisson distribution which is therefore used to predict the number of *C. burnetii* in a random cumulative daily portion of raw milk.

Equation 3 $$\boldsymbol{Raw\_Milk\_Exposure_{person/day} = Poisson(Mean\_Raw\_Milk\_Exposure_{person/day})}$$

Only variability is considered within the model. Uncertainty will be investigated in sensitivity scenarios.

2.1 ***Baseline model***

The baseline assumptions reflect the general endemic status of *C. burnetii* in cattle in the UK, although a worst case assumption is made regarding the duration of shedding by an infected cow (see below).

2.2 ***Scenario analysis for an outbreak scenario***

Outbreaks of *C. burnetii* infection resulting in abortion storms typically occur in goats and sheep and not in cattle. This could, in part, be because sheep and goats are seasonal breeders so abortions would occur at the same time while cattle calve all year. There has never been an abortion outbreak in cattle in GB from *C. burnetii* (R. Mearns AHVLA). Indeed, even in The Netherlands massive outbreaks have occurred in goats, but not in cattle. It would be well-documented if abortion storms were occurring in cattle in GB.

As part of the scenario analysis, a hypothetical outbreak scenario in cattle is modelled here. This gives an indication of how the exposures in milk could increase with increased within herd prevalence and increased numbers of infected animals shedding in milk.

2.3 ***Sensitivity analysis***

In Section 5, the values for some of the parameters are changed to assess the impact on the predicted exposures. The volume of milk produced per cow per day (V_i) and the probability that the animal is lactating ($p_{Lactating}$) are not changed because these parameters are relatively well understood. The herd size (H) is based on empirical data and is not therefore changed.

One source of uncertainty is the duration of shedding of *C. burnetii* in milk by an infected cow. The baseline risk assessment is worst case in assuming that an infected cow that is shedding in milk does so every day of the year that it is lactating. A scenario analysis is undertaken in Section 5.1 to investigate the effect of assuming that shedding lasts for a period of one month in a year.

The baseline risk assessment uses data from naturally infected cows on levels of *C. burnetii* in milk in the study of Enright et al. (1959). However, there is some evidence that the maximum level could be considerably higher than the values in that study and for this reason a sensitivity analysis with a higher maximum is undertaken in Section 5.2. Other sensitivity analyses presented in Section 5 including doubling the daily milk consumption, assuming all herds are positive, assuming all cows within a positive herd are infected and assuming all cattle in England and Wales are infected.

3. PARAMETER ESTIMATION

A description of the data and methods used to estimate each parameter described in Section 2 is given in this section for the baseline model and the outbreak scenario.

3.1 ***Parameter estimation for baseline risk assessment***

3.1.1 *Number of cows per herd*

As described in Section2 each cow within a random herd is simulated in terms of its Q fever (infection, shedding) and lactation status. FSA provided data on the number of cows per herd for 81 herds which supply raw milk for human consumption in England and Wales. The total number of cows on those farms was 7,011. The distribution is highly skewed with many small herds and a few very large herds. The maximum herd size was 300 animals and the average herd size was 86.6 animals. Therefore the whole data set comprising all 81 herds was used to define a discrete random variable for herd size H with the probabilities defined by the frequencies shown in Figure 2.

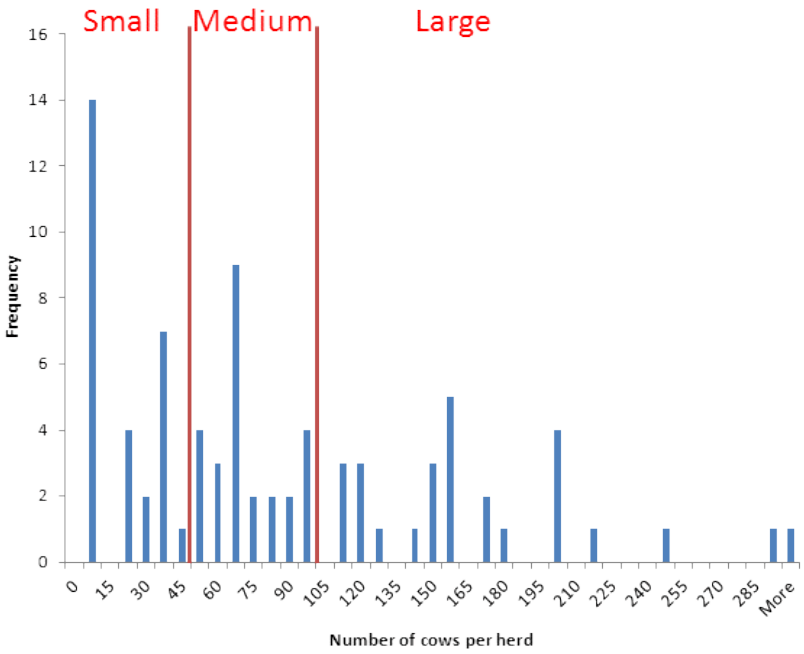

Figure 2: Histogram for number of dairy cattle per herd for the 81 herds supplying raw milk for human consumption in England and Wales.

3.1.2 *Herd prevalence of Coxiella burnetii in UK cattle*

Valergakis et al. (2012) report the prevalence of *C. burnetii* infection in 155 dairy cattle herds in south-west England using a commercial real-time PCR assay. Similarly Paiba et al. (1999) report ELISA data for BTM in GB. However, their data are not used here because they sampled the bulk tank milk itself rather than the animals in the herd. McCaughey et al. (2010) present seroprevalence data for cattle herds in Northern Ireland. The % positive herds varied depending on the type and size of herd. In particular, animal seropositivity was correlated with dairy herd (compared to beef herd) and large herd size. The data of Valergakis et al. (2012) and Paiba et al. (1999) being for BTM can be used for validation.

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

Size of herd	Number of animals	Positive herds/total herds	Proportion positive herds (p_{Herd})
Small	<50	7/22	0.318
Medium	50 – 100	21/35	0.600
Large	>100	50/64	0.781
All dairy herds	--	78/121	0.645

The data for the proportion of positive herds in Table 2 are used as the probabilities (p_{Herd}) in the binomial distribution for the variable, *Herd_Infected*. The appropriate value of p_{Herd} is selected given the simulated herd size, H, according to the estimates in Figure 2:-

$$pp_{Herd} = \begin{cases} 0.318 \ \ if H < 50 \\ 0.600 \ if \ 50 \ \leq H \leq 100 \\ 0.781 \ if \ H > 100 \end{cases}$$

3.1.3 *Probability cow is lactating*

The dry period is an important resting period for the dairy cow, where fresh udder tissue is formed in readiness for the lactation (Dairy Co Technical Information 2012). The lactation period is from calving until the cow has dried up and is the period in which the cow produces milk. This period is usually 300 to 305 days (43 weeks) with limits of 265 to 340 days (38 to 49 weeks) (ARC 2013). There is no seasonality in calving and therefore cows' milk is produced throughout the year in GB overall. There are no seasonal parameters in the model and seasonality is therefore not considered further.

A BetaPert distribution with a minimum of 265 days, a maximum of 340 days and a most likely uniformly distributed between 300 and 305 days was used to determine the number of days per year that an individual cow is lactating. That number was then divided by 365 days to give the probability of lactating (i.e. being milked). This is $p_{Lactating}$ and is calculated as:

$$p_{Lactating\_i} = BetaPert(265, Uniform(300{,}305), 340)/365$$

The model does not allow for the fact that on a farm a given cow is taken out of lactation for consecutive days (dry period), and simply predicts on a daily basis whether each cow is lactating. Given the independent nature of the parameters in the model, this assumption would not affect the output of the model.

3.1.4 *Volume of milk produced per cow per day*

The milk yields per cow per day for each month for the years 2008/9 and 2010/11 were obtained from Kingshay (2013). A Normal distribution with mean 25.6 litres/cow/day and standard deviation 1.263 was used to simulate the volume of milk produced per lactating cow per day. The volume of milk, V_i, in litres per cow per day produced by each individual cow is simulated by:

$$V_i = Normal(\mu = 25.6, \sigma = 1.263)$$

3.1.5 ***Within herd prevalence: Probability animal is positive given herd is positive***

In a naturally infected cattle herd in north-west France, Rodolakis et al. (2007) reported that 37 of 95 female cows tested by PCR were positive for *C. burnetii* DNA. This represents a within herd prevalence of 38.9%. The cows were defined as positive if they were shedding in the vaginal mucus, faeces or milk. McCaughey et al. (2010) found lower within herd prevalences in dairy cattle in Northern Ireland (Table 3). Furthermore these are broken down according to herd size. Since the data from McCaughey et al. (2010) are for the UK, they are used here in the baseline model, with the higher percentage of Rodolakis et al. (2007) being used in the outbreak scenario simulation (Section 4.2) for all dairy herd sizes.

Table 3: Seroprevalence at animal level by herd size for dairy herds in Northern Ireland. Data from McCaughey et al. (2010).

Herd Size	Number of animals	Positive animals	Total animals	Proportion positive (p_{Within_Herd}) used in model
Small	<50	13	380	0.034
Medium	50 – 100	71	696	0.102
Large	>100	160	1280	0.125
All dairy herds	--	244	2356	0.104

The data for the proportion of positive animals in Table 3 are used as the probabilities for within herd infection (p_{Within_Herd}) in the binomial distribution for the variable, *Animal_Infected*. The appropriate value of p_{Within_Herd} is selected given the simulated herd size, H, according to data in Table 3 as set out in:-

$$p_{Within\_Herd} = \begin{cases} 0.034 \ if H < 50 \\ 0.102 \ if \ 50 \ \leq H \leq 100 \\ 0.125 \ if \ H > 100 \end{cases}$$

3.1.6 Probability that an infected animal is shedding on a given day

Contrary to expectation, for all ruminant species, Rodolakis et al. (2007) reported that the shedding of *C. burnetii* could not be related to parturition. This is supported by Rousset et al (2009) who found no significant differences in the proportions of *C. burnetii* shedders between aborting and non-aborting goats. This is important for the risk assessment as it means that data from different papers do not need to account for the abortion status of the animal. Given an animal is infected the probability that it is shedding *C. burnetii* in milk on a given day depends on two factors:-

1) The proportion of infected animals that excrete the pathogen in milk; and
2) The length of time (duration) of shedding the pathogen in milk. This includes whether the animal is a persistent shedder.

The proportion of infected animals that excrete the pathogen in milk

There is considerable variation in the probability that a cow is shedding in milk given it is infected. For example, Rodolakis et al. (2007) reported that 92% of PCR-positive cows were shedding in milk as detected by PCR. However, Guatteo et al. (2012) reported much lower proportions in 24 cows which had experienced an abortion due to *C. burnetii*. Thus, the numbers of cows excreting in milk at 14, 21 and 28 days after the abortion were 9/24, 6/24 and 7/24, respectively (assuming a PCR Ct value >40 is a negative). For the baseline risk assessment it is assumed that the probability ($p_{Shedding}$) that an infected cow is 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. The data of Rodolakis et al. (2007) is used in the outbreak scenario model (Section 3.2).

The length of time (duration) of shedding the pathogen in milk.

According to Guatteo et al. (2012), the PCR Ct values did not change much over the 14 day period, although most cows did not excrete positive milk samples on all of the three days and are therefore intermittent shedders (not shown). Guatteo et al. (2012) write that three cows were identified as persistent shedders in that they were shedding relatively high levels on all three days. 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 long periods of time. They found that the milk of four positive cows was still positive 205 days later after each had calved, and one of the animals was found to be still shedding 1,000 infective guinea pig doses per 2 ml of milk. Serologic evidence indicated that this animal was infected with the organism of Q fever at least 405 days prior to this second milk sampling. *C. burnetii* could not be found in the milk of the other three cows at the time point of the second milk sampling. For the purpose of risk assessment it is assumed that a cow which does shed *C. burnetii* does so for the whole year in the baseline model. Since the model calculates the exposure on a

daily basis, the duration of shedding is not included specifically in the model. Instead it is included in the probability of shedding ($p_{Shedding}$) in this model.

3.1.7 Number of Coxiella burnetii present in raw milk

Enright et al. (1957) used a bioassay approach to measure *C. burnetii* in raw cows' milk. Two consecutive passages were done in guinea pigs to allow for antibody generation from dead bacteria in the first assay and so the results do represent viable bacteria. The unit of *C. burnetii* infectivity is the intraperitoneal guinea pig infective dose 50% ($GP\_IP\_ID_{50}$). The number of $GP\_IP\_ID_{50}$ in milk was estimated by serial dilution such that half of the guinea pigs given a certain dilution through the intraperitoneal route were infected. Enright et al. (1957) found that the milk from 18 of 137 individual cows in a dairy herd contained viable *C. burnetii*. Titration of those positive milk samples from those 18 shedding cows showed three contained 1,000, five contained 100, five contained 10 and five contained one $GP_IP_ID_{50}$s per 2 ml (Table 4). These are presented as a histrogram in Figure 3.

Table 4: Quantitative data for *C. burnetii* infectivity reported in cows' milk by Enright et al. (1957)*.

	Samples	Guinea pig intraperitoneal infective doses 50% ($GP_IP_ID_{50}$s) per 2 ml of milk
18 positive cows from a naturally infected herd	3 cows	1,000
	5 cows	100
	5 cows	10
	5 cows	1
	Average	197.5 per 2 ml

*Enright et al. (1957) reported a maximum of 10,000 per 2 ml of milk from one cow which they experimentally infected through inoculation of the teat. They also cite other experiments which reported 10,000 per ml. This is equivalent to 20,000 per 2 ml but is omitted from the distribution fitting here because the cows were not naturally infected. In Section 5.2, a sensitivity analysis is undertaken using a distribution fitted including the 20,000 per 2 ml data point.

The Normal distribution (Figure 3) gives a good fit to the $\log_{10}$-transformed data and $\chi^2 = 0.667$ (1 df) which is not significant (P = 0.88). Thus the null hypothesis that the observed data and fitted distribution are the same is not rejected and a Normal distribution is used to model the number of *C. burnetii* per ml of milk, C_{ml}, given the animal is shedding:

Equation 4 $$C_{ml} = 0.5 \times 10^{Normal(\mu=1.333,\sigma=1.0847)}$$

where μ and σ represent the mean and standard deviation for the $\log_{10}$-transformed counts for *C. burnetii* ID_{50} units per 2 ml of milk (which was positive for *C. burnetii*) based on data in Table 4. In effect it is assumed that the numbers of *C. burnetii* per ml of milk from different shedding cows are lognormal in distribution.

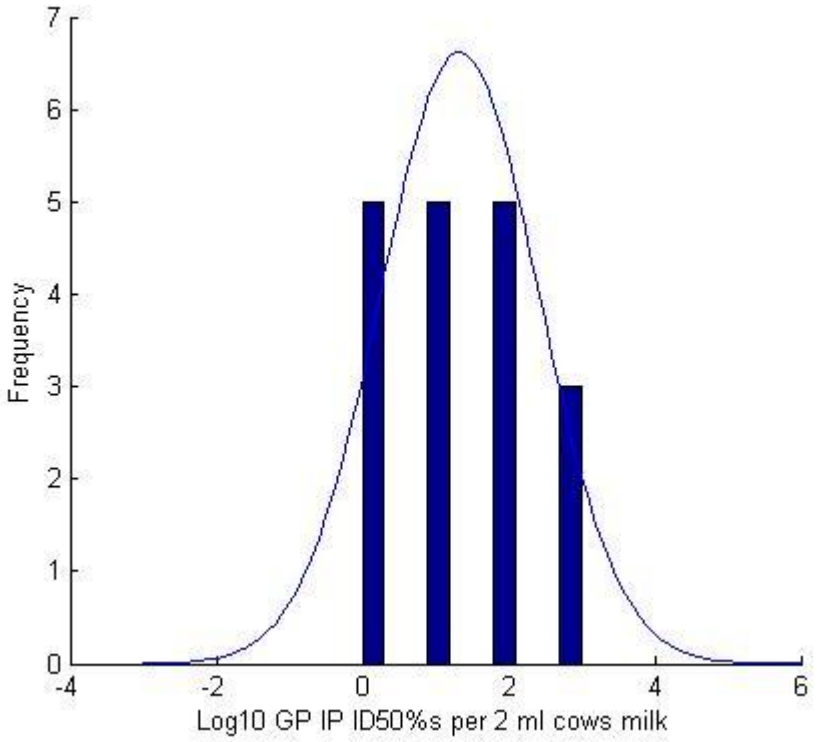

Figure 3: Fitted normal distribution for $\log_{10}$-transformed numbers of GP_IP_ID_{50}s per 2 ml of milk from 18 naturally-infected cows which are shedding *C. burnetii* in milk, i.e. positives only (data in Table 4).

3.1.8 *Daily consumption of milk*

The mean consumption for milk used in the baseline risk assessment is 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 included males and females and, importantly, consumers only, (i.e. the lower figures based on both consumers and non-consumers are not used). Raw milk delivery companies in England[1] appear to sell only whole unpasteurised milk with no mention of skimmed and/or semi-skimmed raw milks. Therefore whole milk consumption was considered to be the most appropriate to undertake an exposure assessment. Although 1.5 - 3 year olds, 4 - 10 year olds and 11 - 18 year old persons drink more whole milk at 0.297 kg, 0.199 kg, and 0.164 kg per person per day, respectively, (Table 1) it is considered unlikely that persons in those age groups would drink raw milk to the total

exclusion of pasteurised milk because they are at school for much of the time. Persons in the 65+ year age group also drink more whole milk at 0.224 kg/person per day. However, there is evidence from a study on tap water consumption (Drinking Water Inspectorate 2008) that much of this will be in hot drinks and hence *C. burnetii* is likely to be inactivated by the high temperature[2]. Using these consumption figures is thus worst case in that it is assumed that the individual person drinks raw milk to the total exclusion of pasteurised milk and that none of this is subjected to any heat treatment or added to a hot drink.

Consumption of semi-skimmed milk (1.8% fat) and 1% fat milk is 0.128 kg and 0.143 kg/person/day for the 19 to 64 year old age group and greater than that for whole milk. However, it is considered unlikely that the raw milk consumed is skimmed or semi-skimmed as discussed above.

Assuming the density of milk is 1.0 kg/l and that raw milk is consumed in the same volumes per person per day as pasteurised whole milk, then the cumulative daily consumption of raw milk ($M_{Litre/Day}$) is 0.127 litres per person.

A proportion of the 0.127 kg/person/day of whole milk consumed by 19 – 64 year olds is likely to be in coffee or tea and in this respect the 0.127 litres/person/day for raw milk in the baseline risk assessment may overestimate the viable *C. burnetii* exposure. For the purpose of sensitivity analysis in Section 5.3, a value of 0.254 litres/person/day is used. This is double the baseline value of 0.127 litres/person/day.

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

A summary of the model parameters, notation and parameterisation is given in Table 5.

[2]60% of daily drink's intake was in the form of hot drinks (coffee, tea, hot milky drink) for the 55+ year age group compared to 46% in 16 to 54 year age groups combined (Drinking Water Inspectorate 2008). While coffee may be consumed without milk, tea is invariably consumed with milk. Some 40% of daily drinks' intake was tea in the 55+ year age group compared to 26.7% for the 16 to 54 year age groups combined. The extra 13.3% drinks' intake amounts to approximately 250 cm^3 per day and equates to just less than a mug (275 cm^3).

Table 5: 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
Number of dairy cows per herd	H	Used empirical data for 81 cattle herds in England and Wales supplying raw milk (Figure 2).	.FSA
Probability herd is positive	p_{Herd}	$p_{Herd} = \begin{cases} 0.318 \ if H < 50 \\ 0.600 \ if \ 50 \ \leq H \leq 100 \\ 0.781 \ if \ H > 100 \end{cases}$	McCaughey et al (2010)
Probability animal is positive given herd is positive	p_{Within_herd}	$p_{Within\_Herd} = \begin{cases} 0.034 \ if H < 50 \\ 0.102 \ if \ 50 \ \leq H \leq 100 \\ 0.125 \ if \ H > 100 \end{cases}$	McCaughey et al (2010)
Probability animal is lactating[1]	$p_{Lactating}$	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	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

[1] Most dairy herds are all year round calving to ensure continuity of milk supply to market but there will be variation and some herds may be batch calving for heifers entering the herd. The assumption is the all year round calving pattern.

3.2 ***Parameter estimation for outbreak scenario***

The changes in the parameters for the outbreak scenario are discussed below.

Probability animal is positive given herd is positive

An abortion storm gives an increase in the number of infected animals within a herd. The outbreak (in goats at least) is linked to increased shedding due to abortion, perhaps after the pathogen is introduced into a naïve herd or a new strain is introduced. However, the storm might only occur in a few herds and for this reason p_{Within_Herd} is increased while p_{Herd} is left unchanged in this simulation. For the baseline risk assessment it is assumed that the probability a cow is positive given the herd is positive (p_{Within_Herd}) is 3.4%, 10.2% or 12.5% for small, medium or large dairy herds, respectively (McCaughey et al 2010). For the purpose of this outbreak scenario, the higher value of 38.9% is used for p_{Within_Herd} reflecting 37 of 95 cows in a dairy herd in France (Rodolakis et al. 2007).

Probability an infected cow is shedding

For the baseline risk assessment it is assumed that the probability a cow is shedding is given by 22/72 ($p_{Shedding}$ = 0.3055) according to the summed data of Guatteo et al. (2012) over days 14, 21 and 28 post abortion. For the purpose of this outbreak scenario, a value for $p_{Shedding}$ = 0.92 from the data of Rodolakis et al (2007) presented in Table 6 is used in the simulation.

Table 6: Proportion of infected cattle that excrete in *Coxiella burnetii* in milk. Taken from Table 4 of Rodolakis et al. (2007)

	Number PCR positive animals in herd*	Number shedding through milk	Proportion
Bovine	37	34	0.92

*The 37 cows were defined as positive if they were shedding in the vaginal mucus, faeces or milk.

Effect on C_{ml}.

The levels of *C. burnetii* shed in milk by a shedding cow are assumed to be the same as for the baseline model. Although there is some evidence of super-shedding in sheep (Porten et al. 2006), there are no quantitative data on post-abortion levels in milk in cattle.

Effect on $p_{Lactating}$

It is known that goats undergoing abortion do not produce much milk until the time when the kid is expected (R. Mearns AHVLA pers comm. based on the largest outbreak in goats investigated in detail by AHVLA in 2010). A case could be made

that $p_{Lactating}$ decreases during an abortion storm as goats which have aborted produce less milk, however there is no information relating to cows. Here $p_{Lactating}$ is left unchanged.

The parameter values used in the outbreak scenario are summarised in Table 9.

Table 7: Summary of values of parameters used in the outbreak scenario compared to baseline

Sensitivity scenario	Parameter	Baseline	Outbreak scenario
Number of dairy cows per herd	H	Used empirical data for 81 cattle herds in England and Wales supplying raw milk (Figure 2).	Unchanged
Probability herd is positive	p_{Herd}	$p_{Herd} = \begin{cases} 0.318 \ if H < 50 \\ 0.600 \ if \ 50 \ \leq H \geq 100 \\ 0.781 \ if \ H > 100 \end{cases}$	Unchanged
Probability animal is positive given herd is positive	p_{Within_herd}	$p_{Within\_Herd} = \begin{cases} 0.034 \ if H < 50 \\ 0.102 \ if \ 50 \ \leq H \geq 100 \\ 0.125 \ if \ H > 100 \end{cases}$	0.389 for all herd sizes reflecting cows in a dairy herd in France (Rodolakis et al. 2007)
Probability animal is lactating[1]	$p_{Lactating}$	Pert (265, Uniform (300,305); 340)/365	Unchanged
Probability animal is shedding *C. burnetii* in milk given animal is lactating and infected	$p_{Shedding}$	22 of 72 infected cows (0.3055)	0.92 based on data from Rodolakis et al. (2007) - Table 6
Volume of milk (per animal per day)	V	Normal (25.6, 1.263)	Unchanged
Coxiella burnetii concentration in milk (shedders)	C_{ml}	Guinea pig intraperitoneal ID_{50} per ml distributed as 0.5 x $10^{Normal\ (1.333,\ 1.0847)}$	Unchanged
Daily consumption of milk	$M_{Litre/Day}$	0.127 litres	Unchanged

*Assumes density of milk is 1 kg/l

4. **RESULTS**

As stated in the previous section, each iteration of the model represents the milk produced from a single herd on a given day. It is implicitly assumed that each consumer takes all their milk from one herd on a given day.

It is assumed that the milk volumes from all the lactating cows in a given herd on a given day are mixed. In this respect, each 127 g portion of milk represents an average from a particular herd on a given day. Overall at least 10,000 iterations were done, each iteration representing one herd-day.

4.1 ***Daily exposure to C. burnetii through consumption of raw milk: Baseline results***

To try to achieve convergence, 500,000 iterations were run for the baseline model. The mean *C. burnetii* concentration for BTM from a given herd ($Mean\_Raw\_Milk_{C.burnetii/litre}$) on a given day was simulated for 500,000 herd-days and each was used to give an exposure per serving using Equation 3. Overall, including both positive and negative exposures, the overall mean level of exposure through raw milk for the baseline model was 532.0 *C. burnetii* $GP\_IP\_ID_{50}$ per person per day, with 2.5 percentile and 97.5 percentile exposures of and 0 and 3,409.8 $GP\_IP\_ID_{50}$ per person per day, respectively.

4.1.1 *Probability of exposure to C. burnetii in raw milk*

In the baseline model, 57.96% of the 500,000 daily exposures per person simulated had zero *C. burnetii*. Thus 42.03% of daily exposures were positive, and the probability of exposure per person to one or more *C. burnetii* through the daily consumption of raw milk is 0.4203.

4.1.2 *The level of exposure per day, given exposure has occurred*

For the 42.03% of daily exposures which were positive for *C. burnetii* the mean was 1,266 $GP\_IP\_ID_{50}$ per person per day. The non-zero exposures (from 1,000 simulated herds) are plotted as a histogram in Figure 4. The 2.5^{th} and 97.5^{th} percentiles were 2 and 7,524 $GP\_IP\_ID_{50}$ per person per day, respectively.

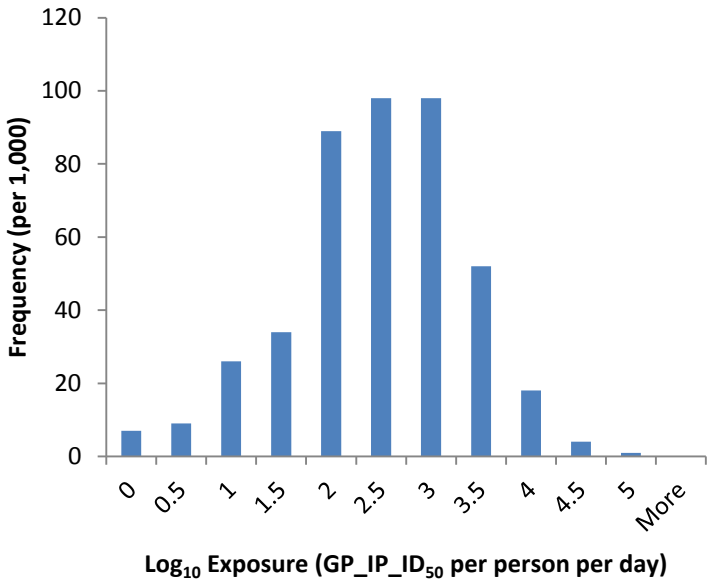

Figure 4: Frequency distribution for log_{10}-transformed positive exposures (per person per day) through consumption of raw milk. 1,000 daily servings simulated under baseline conditions for purpose of graphical presentation.

4.2 ***Daily exposure to C. burnetii through raw milk: Outbreak scenario***

Overall, including the positive and negative exposures, the mean exposure for the outbreak scenario model was 6,331 *C. burnetii* $GP\_IP\_ID_{50}$ per person per day through consumption of raw milk, with 2.5 percentile and 97.5 percentile exposures of 0 and 34,217 $GP\_IP\_ID_{50}$ per person per day, respectively.

4.2.1 *Probability of exposure to C. burnetii in raw milk*

In the outbreak scenario model, 45.47% of 10,000 daily exposures (per person) simulated had zero *C. burnetii*. Thus 54.53% of daily exposures (per person) were positive, and the probability of exposure to one or more *C. burnetii* through daily consumption of raw milk is 0.545 per person. Compared to the baseline model there has been only a small change in this probability, which is due to the assumption that the parameter p_{Herd} is unchanged in the outbreak scenario here.

4.2.2 *The level of exposure in a serving, given exposure has occurred*

For the 54.53% of daily exposures which were positive for *C. burnetii* the mean was 11,612 $GP\_IP\_ID_{50}$ per person per day, which is a nine-fold increase compared to the baseline of 1,266 $GP\_IP\_ID_{50}$ per person per day. The non-zero exposures (from 1,000 simulated herds) are plotted as a histogram in Figure 5. The modal exposure in Figure 5 is 10^4 $GP\_IP\_ID_{50}$ per person per day. The 2.5^{th} and 97.5^{th} percentiles were 342 and 54,605 $GP\_IP\_ID_{50}$ per person per day, respectively.

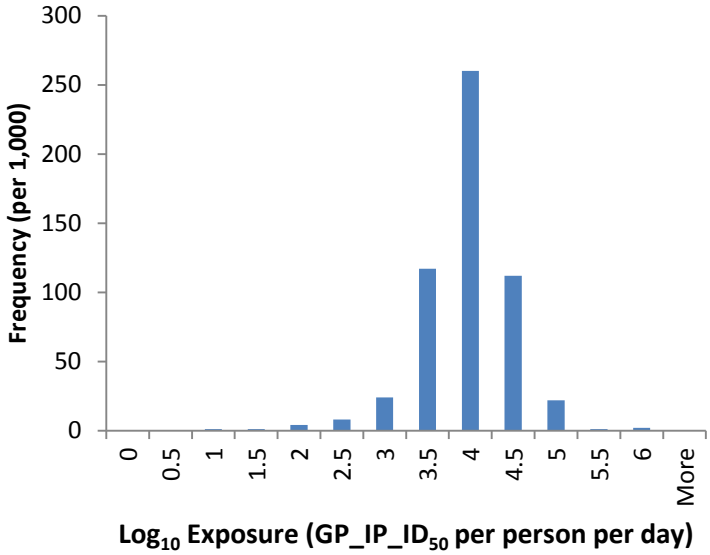

Figure 5: Frequency distribution for log_{10}-transformed positive exposures (per person per day) through consumption of raw milk. 1,000 daily servings simulated under outbreak scenario conditions for purpose of graphical presentation.

4.3 ***Validation of model through simulation of levels of C. burnetii per ml of raw milk in the bulk tank***

From the baseline simulation, the mean level of *C. burnetii* is 4,189.0 $GP\_IP\_ID_{50}$ per litre of raw milk from the bulk tank with 2.5th and 97.5th percentiles in the simulation of 0 and 26,848 $GP\_IP\_ID_{50}$ per litre, respectively. These seemingly high values reflect the values of 1,000 $GP_IP_ID_{50}$s per 2 ml for three raw milk samples in the data (Table 4) to which the log-Normal distribution, used in the simulation here, was fitted (Figure 3). According to Valergakis et al. (2012) some 13.5% of BTM milk samples contained >4.0 log qPCR units/ml (see Figure 6). For comparison, the distribution for the number of *C. burnetii* $GP\_IP\_ID_{50}$ per ml of BTM milk as simulated is presented in Figure 7. 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 in Figure 6. The two distributions are similar *in shape* with each having two peaks. The zero peak reflects negative herds and 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 lower by some three logs compared to the qPCR data (Figure 6). Indeed the histogram for qPCR results for the number of *C. burnetii* per ml of BTM (Figure 6) as published by Valergakis et al. (2012) for south west England shows some 66% of samples with ≥3.0 log qPCR units/ml, while the 97.5th percentile simulated is 1.42 log_{10} $GP_IP_ID_{50}$s /ml (Figure 7). In Table 8, the mean number of qPCR units in the BTM of Valergakis et al. (2012) is estimated at 7,361/ml. This is based on those 13.5% of BTM samples recorded as >4 log in Figure 6 having an assumed value 4.5 log qPCR units/ml. This is equivalent to an arithmetic mean of 7,361,000 qPCR units/litre,

some 1,800-fold higher than the 4,189 GP_IP_ID_{50}s per litre BTM in the simulation. Thus initial consideration would suggest that the model is underestimating the levels of *C. burnetii* in BTM by at least three orders of magnitude compared to PCR data obtained from BTM in the south-west of England. This apparent discrepancy is reconciled in Section 6.

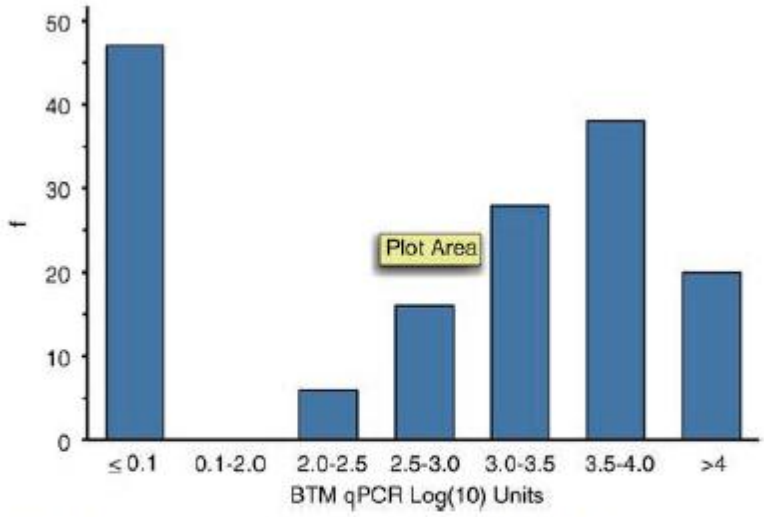

FIG 1: Histogram showing frequency of real-time PCR results for *Coxiella burnetii* in dairy herds (n=155) in south-west England, expressed as $\log_{10}$ of the number of *C burnetii* per ml of bulk tank milk

Figure 6: Quantitative PCR results for *C. burnetii* DNA in BTM (Valergakis et al. 2012)

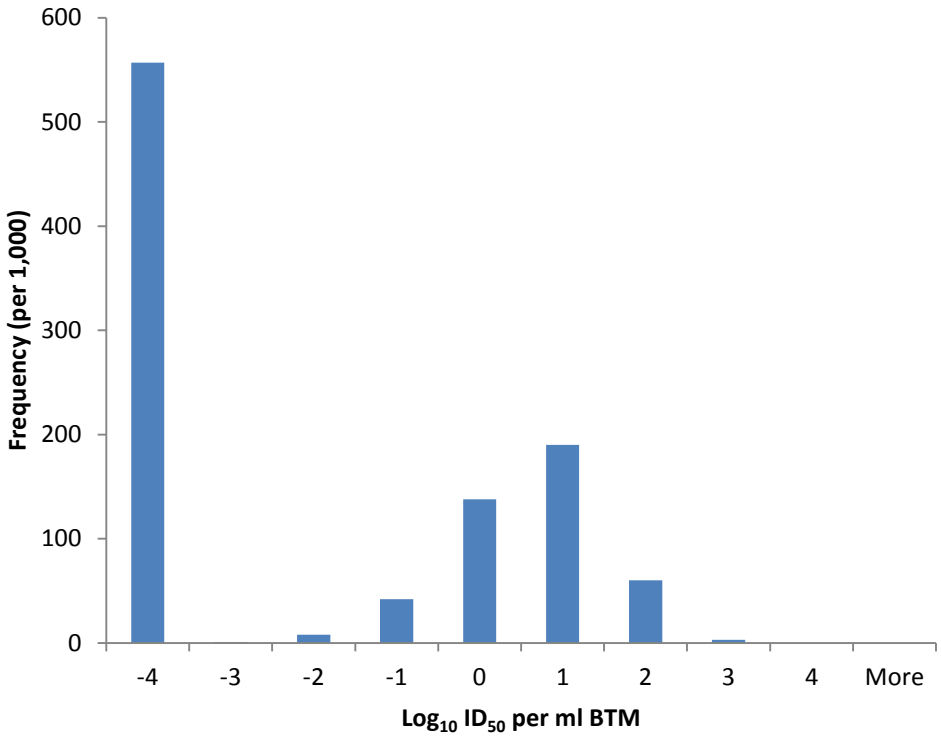

Figure 7: Simulated GP_IP_ID_{50}s of *Coxiella burnetii* per ml of raw BTM milk plotted on a log scale for comparison with Figure 6. 1,000 samples simulated under baseline conditions for purpose of graphical presentation; zero per ml represented as -4.

Table 8: Estimation of mean qPCR units per ml of BTM using data from Valergakis et al. (2012).

Frequency	log qPCR units/ml	qPCR units per ml	Freq x qPCR units
47	0.1	1.3	59.17
6	2.5	316.2	1897.4
17	3	1000.0	17000.0
27	3.5	3162.3	85381.5
38	4	10000.0	380000.0
21	4.5	31622.8	664078.3
Total = 156			Sum = 1148416.3
Mean = 7,361.6 qPCR units/ml			

5. SENSITIVITY ANALYSIS

The parameter values used in the sensitivity analyses are summarised in Table 9.

Table 9: Summary of values of parameters used in sensitivity analyses compared to baseline

Sensitivity scenario	Parameter	Baseline	Sensitivity analysis
Duration of shedding	$p_{Shedding}$	0.3055	0.0255
Including maximum of 20,000 GP_IP_ID_{50}s per 2 ml of raw milk	C_{ml}	Guinea pig intraperitoneal ID_{50} per ml distributed as $0.5 \times 10^{Normal\ (1.333,\ 1.0847)}$	Guinea pig intraperitoneal ID_{50} per ml distributed as $0.5 \times 10^{Normal\ (1.4895,\ 1.2549)}$
Doubling daily milk consumption size	$*M_{Litre/day}$	0.127 kg/person/day	0.254 kg/person/day
Assuming all herds positive	p_{Herd}	$\begin{cases} 0.318\ if H < 50 \\ 0.600\ if\ 50\ \leq H \leq 100 \\ 0.781\ if\ H > 100 \end{cases}$	1.0 for all herds
Assuming all cows within a positive herd are infected	p_{Within_herd}	$\begin{cases} 0.034\ if H < 50 \\ 0.102\ if\ 50\ \leq H \leq 100 \\ 0.125\ if\ H > 100 \end{cases}$	1.0 for all herd sizes
Assuming all cattle infected	p_{Herd} and p_{Within_herd}	See above	Both set to 1.0 for all herd sizes

*Assumes density of milk is 1 kg/l

5.1 ***Sensitivity analysis: Duration of shedding***

The baseline risk assessment assumes that the duration of shedding of *C. burnetii* by an infected cow is infinite, i.e. that a cow which is positive and shedding in milk is permanently shedding. That assumption reflects the lack of data on the duration of shedding in milk by infected cows (Section 3.1.6).

5.1.1 *Parameter estimation for scenario analysis for duration of shedding*

Here a "What If" scenario is conducted to assess the effect of an infected cow only shedding for one month per year. Thus the value of $p_{Shedding}$ in Equation 1 is divided by 12 and a value of 0.0255 is used. As discussed in Section 3.1.6, the model

calculates the exposure on a daily basis. Although the duration of shedding is not included specifically in the model it is linked to the probability of shedding.

5.1.2 *Results for overall exposures*

To try to achieve convergence, 50,000 iterations were run. Overall, including the positive and negative exposures, the mean level of exposure for the scenario with shorter duration of shedding through consumption of raw milk was 12-fold lower than that for the baseline model (532.0) at 43.1 *C. burnetii* GP_IP_ID_{50} per person per day, with 2.5 percentile and 97.5 percentile exposures of 0 and 126 GP_IP_ID_{50} per person per day, respectively.

5.1.3 *Probability of exposure to C. burnetii in raw milk*

In the scenario with shorter duration of shedding, 89.5% of 50,000 daily exposures simulated had zero *C. burnetii*. Thus 10.5% of daily exposures were positive, and the probability of exposure to one or more *C. burnetii* through consumption of raw milk was reduced four-fold compared to the baseline model from 0.4203 per person day to 0.1048 per person per day.

5.1.4 *The level of exposure per person per day, given exposure has occurred*

For the 10.5% of daily exposures which were positive for *C. burnetii* the mean was 411.5 GP_IP_ID_{50} per person per day and roughly a third of that in the baseline line model (1,266 GP_IP_ID_{50} per person per day). The non-zero exposures (from 1,000 simulated herds) are plotted as a histogram in Figure 8 and are lower than those for the baseline model (Figure 4). The 2.5th and 97.5th percentiles were 1 and 2,290 GP_IP_ID_{50} per person per day, respectively.

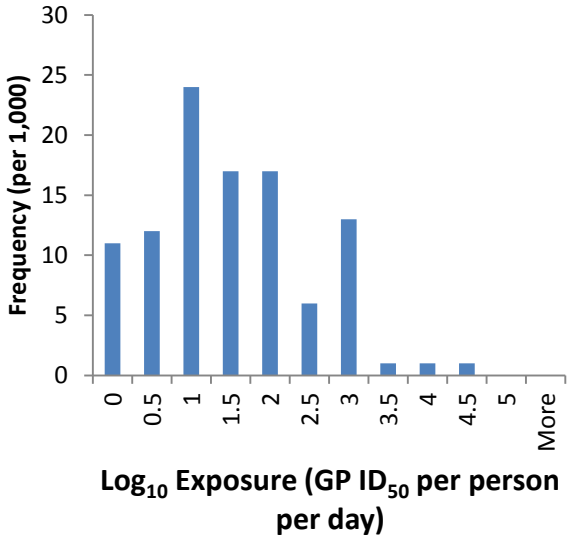

Figure 8: Frequency distribution for log_{10}-transformed positive exposures (per person per day) through raw milk. 1,000 daily servings simulated under low shedding scenario conditions for purpose of graphical presentation.

5.2 ***Sensitivity analysis: Including a maximum of 20,000 GP_IP_ID$_{50}$ per 2 ml of milk***

Enright et al. (1957) reported that the milk of an experimentally infected dairy cow contained 10,000 GP_IP_ID$_{50}$s of *C. burnetii* (presumably this is per 2 ml). The animal had been infected by introducing the Henzerling strain of the organism into the teat canal. The positive milk sample was obtained on the ninth day after inoculation. Enright et al. (1957) note that information from other investigators (not cited) revealed that 10,000 GP_IP_ID$_{50}$s per ml of milk was the maximum. That is equivalent to 20,000 GP_IP_ID$_{50}$s per 2 ml.

5.2.1 *Parameter estimation for scenario analysis*

For the purpose of fitting a Normal distribution to the log-transformed *C. burnetii* loadings in this sensitivity analysis, a maximum of 20,000 per 2 ml was used in addition to the 18 data points in in Table 4. Including the 20,000 per 2 ml maximum improved the fit of Normal distribution (χ^2 statistic = 0.158 1 df, P = 0.98) to the log-transformed data compared to that used in the baseline model. The frequency distribution is shown in Figure 9. The mean, μ, and standard deviation, σ, increase from 1.333 and 1.0847 to 1.4895 and 1.2549 respectively. In the sensitivity analysis, C_{ml}, the number of guinea pig intraperitoneal ID$_{50}$ per ml of milk form shedding cows is distributed as:

Equation 5

$$C_{ml} = 0.5 \times 10^{Normal(\mu=1.4895,\sigma=1.2549)}$$

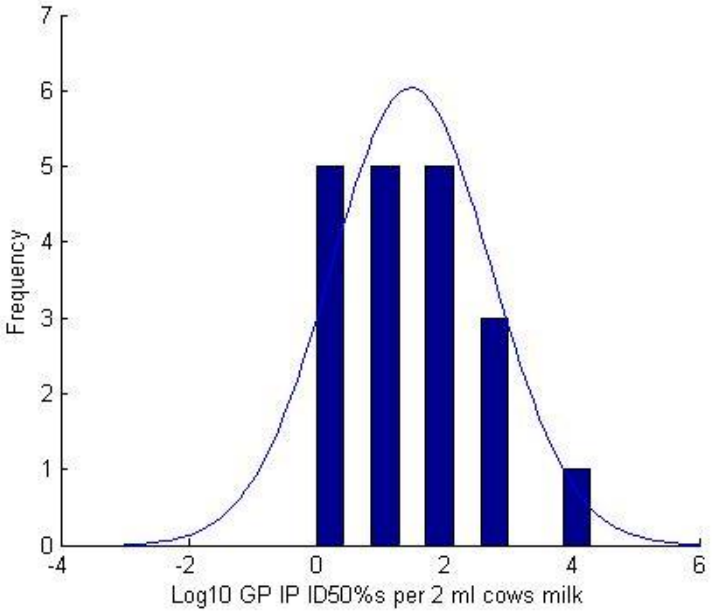

Figure 9: Fitted normal distribution for log$_{10}$-transformed numbers of GP_IP_ID$_{50}$s per 2 ml of milk from shedding cows (data for 18 samples in Table 4) plus an additional point with 20,000 GP_IP_ID$_{50}$s per 2 ml (see text).

5.2.2 *Results for overall exposures*

Overall, including the positive and negative exposures, the mean level of exposure in the scenario including the maximum of 20,000 GP_IP_ID_{50} per 2 ml of raw milk was five-fold higher than the 532.0 GP_IP_ID_{50} per person per day for the baseline model at 2,777.5 *C. burnetii* GP_IP_ID_{50} per person per day, with 2.5 percentile and 97.5 percentile exposures of 0 and 9,412 GP_IP_ID_{50} per person per day, respectively.

5.2.3 *Probability of exposure to C. burnetii in raw milk*

In the scenario including the maximum of 20,000 GP_IP_ID_{50} per 2 ml of milk, 58.77% of the 10,000 daily exposures per person simulated had zero *C. burnetii*. Thus 41.22% of daily exposures were positive, and the probability of exposure to one or more *C. burnetii* per person per day through consumption of raw milk was little affected compared to the baseline model, being decreased slightly from 0.4203 to 0.4122. This slight decrease could reflect the larger standard deviation used in the Normal distribution namely 1.2549 compared to 1.0847 in the baseline model. Thus, as the standard deviation increases there will be fewer positive samples, albeit with higher levels of *C. burnetii* in those positives.

5.2.4 *The level of exposure per person per day, given exposure has occurred*

For the 41.22% of daily exposures which were positive for *C. burnetii*, the mean was 6,736.7 GP_IP_ID_{50} per person per day and roughly five times greater than that in the baseline line model (1,266 GP_IP_ID_{50} per person per day). The 2.5^{th} and 97.5^{th} percentiles were 3 and 22,359 GP_IP_ID_{50} per person per day, respectively.

5.3 ***Sensitivity analysis: Doubling the daily consumption of raw milk***

5.3.1 *Parameter assumption for scenario analysis for doubling serving size*

The daily consumption per person for (raw) milk was doubled from 127 g to 254 g per person per day.

5.3.2 *Results for overall exposures*

Overall, including the positive and negative exposures, the mean level of exposure in the scenario in which the daily raw milk consumption was 254 g per person per day was almost double the value of 532.0 for the baseline model at 1,016.9 *C. burnetii* GP_IP_ID_{50} per person per day, with 2.5 percentile and 97.5 percentile exposures of 0 and 6,145 GP_IP_ID_{50} per person per day, respectively.

5.3.3 *Probability of exposure to C. burnetii in raw milk*

In the scenario with double the milk consumption, 57.71% of the 10,000 daily exposure simulated had zero *C. burnetii*. Thus 42.29% of exposures were positive, and the probability of exposure to one or more *C. burnetii* through consumption of

raw milk was little affected compared to the baseline model, being increased slightly from 0.4203 per person per day to 0.4229.

5.3.4 *The level of exposure in a serving, given exposure has occurred*

For the 42.29% of daily exposures which were positive for *C. burnetii* the mean was 2,404.6 GP_IP_ID_{50} per person per day and roughly double that in the baseline model (1,266 GP_IP_ID_{50} per person per day). The 2.5^{th} and 97.5^{th} percentiles were 4 and 13,517 GP_IP_ID_{50} per person per day, respectively.

5.4 ***Sensitivity analysis: Assuming all herds are infected***

5.4.1 *Parameter assumptions for scenario analysis in which all herds are infected*

The between herd prevalences (p_{Herd}) are set to 1.0 for all three herd sizes.

5.4.2 *Results for overall exposures*

Overall, including the positive and negative exposures, the mean level of exposure in the scenario in which all herds were positive was increased by almost 1.5-fold relative to the value of 532.0 for the baseline model at 778.3 *C. burnetii* GP_IP_ID_{50} per person per day, with 2.5 percentile and 97.5 percentile exposures of 0 and 5,180 GP_IP_ID_{50} per person per day, respectively.

5.4.3 *Probability of exposure to C. burnetii in raw milk*

In the scenario with all herds positive, 37.29% of the 10,000 daily exposures per person simulated had zero *C. burnetii*. Thus 62.71% of daily exposures were positive, and the probability of exposure to one or more *C. burnetii* through consumption of raw milk compared to the baseline model was increased by 1.5-fold from 0.4203 to 0.6271 per person per day.

5.4.4 *The level of exposure per person per day, given exposure has occurred*

For the 62.71% of daily exposures which were positive for *C. burnetii* the mean was 1,241.2 GP_IP_ID_{50} per person per day and very similar to that in the baseline line model (1,266 GP_IP_ID_{50} per person per day). The 2.5^{th} and 97.5^{th} percentiles were 2 and 7,940 GP_IP_ID_{50} per person per day, respectively.

5.5 ***Sensitivity analysis: Assuming all cows within a positive herd are infected***

5.5.1 *Parameter assumptions for scenario analysis in which all cattle within a positive herd are infected*

The within herd prevalences (p_{Within_herd}) are set to 1.0 for all three herd sizes.

5.5.2 *Results for overall exposures*

Overall, including the positive and negative exposures, the mean level of exposure through consumption of raw milk in the scenario in which all cattle within a positive

herd were infected was increased nine-fold relative to the value of 532.0 for the baseline model at 4,837.5 *C. burnetii* GP_IP_ID_{50} per person per day, with 2.5 percentile and 97.5 percentile exposures of 0 and 28,674 GP_IP_ID_{50} per person per day, respectively.

5.5.3 *Probability of exposure to C. burnetii in raw milk*

In the scenario with all cattle infected within a positive herd, 45.76% of the 10,000 daily exposures simulated had zero *C. burnetii*. Thus 54.24% of daily exposures were positive, and the probability of exposure to one or more *C. burnetii* through a serving of raw milk compared to the baseline model was increased by 1.3-fold from 0.4203 per person per day to 0.5424.

5.5.4 *The level of exposure in a serving, given exposure has occurred*

For the 54.24% of daily exposures per person which were positive for *C. burnetii* the mean was 8,918.3 GP_IP_ID_{50} per person per day and about seven-fold higher than that in the baseline line model (1,266 GP_IP_ID_{50} per person per day). The 2.5^{th} and 97.5^{th} percentiles were 240 and 45,743 GP_IP_ID_{50} per person per day, respectively.

5.6 ***Sensitivity analysis: Assuming all cattle in England and Wales are infected***

5.6.1 *Parameter assumptions for scenario analysis in which all cattle are infected*

The between herd prevalences (p_{Herd}) and the within herd prevalences (p_{Within_herd}) are set to 1.0 for all three herd sizes.

5.6.2 *Results for overall exposures*

Overall, including the positive and negative exposures, the mean daily level of exposure through consumption of raw milk in the scenario in which all cattle were positive was increased 18-fold relative to the value of 532.0 for the baseline model at 9,616.7 *C. burnetii* GP_IP_ID_{50} per person per day, with 2.5 percentile and 97.5 percentile exposures of 0 and 49,644 GP_IP_ID_{50} per person per day, respectively.

5.6.3 *Probability of exposure to C. burnetii in raw milk*

In the scenario with all herds positive, only 4.05% of the 10,000 daily exposures simulated had zero *C. burnetii*. Thus 95.95% of the daily exposures were positive, and the probability of exposure to one or more *C. burnetii* through consumption of raw milk compared to the baseline model was increased by 2.3-fold from 0.4203 per person per day to 0.9595.

5.6.4 *The level of exposure in a serving, given exposure has occurred*

For the 95.95% of daily exposures per person which were positive for *C. burnetii* the mean was 10,022.2 GP_IP_ID_{50} per person per day and eight-fold higher than that in

the baseline line model (1,266 GP_IP_ID_{50} per person per day). The 2.5th and 97.5th percentiles were 110 and 50,412 GP_IP_ID_{50} per person per day, respectively.

5.7 ***Comparison of sensitivities***

The results of the sensitivity analyses described above are summarised in Table 10. The frequency distributions for the positive daily exposure in the baseline, outbreak and low shedding scenarios are compared in Figure 10.

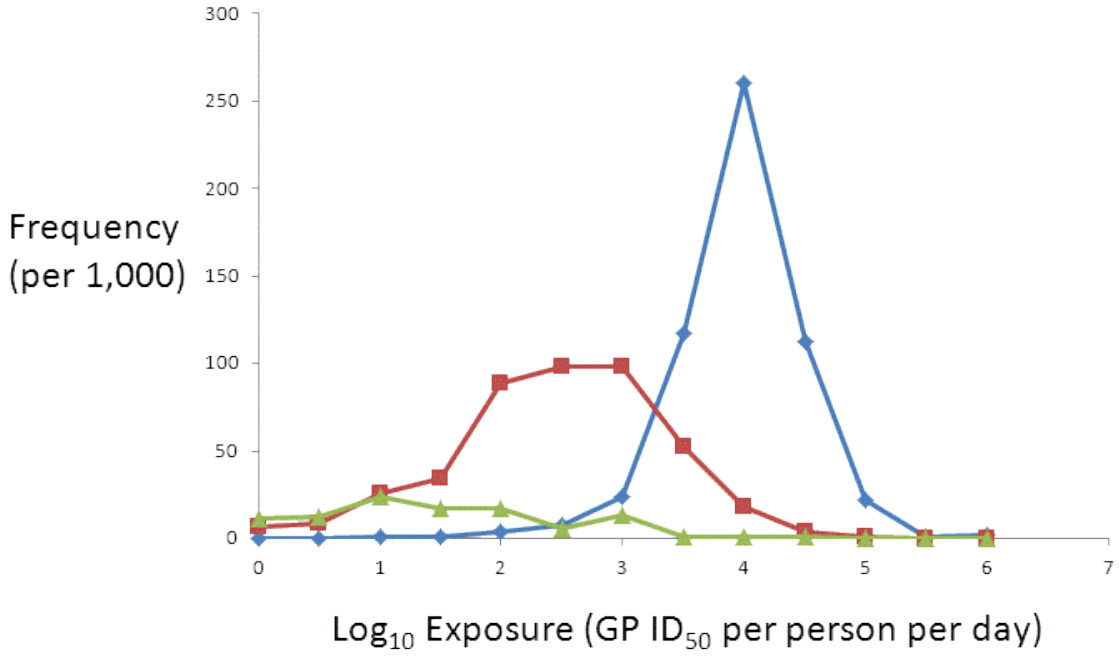

Figure 10: Comparison of frequency distributions for log_{10}-transformed positive exposures through cumulative daily consumption of raw milk – baseline from Figure 4 (■), outbreak scenario from Figure 5 (♦) and low shedding scenario from Figure 8 (▲).

In terms of the sensitivity, the factors investigated which had the biggest impact on total exposure over the population were the duration of shedding and the within herd prevalence. Thus reducing the duration of shedding from 12 months to 1 month decreased the overall mean level of exposure (including both negative and positive exposures) by 12-fold. Increasing the within herd prevalence to 1.0 (such that all cows in a positive herd were infected) increased the overall mean level of exposure (including both negative and positive exposures) by nine-fold. Including a raw milk sample with 20,000 GP_IP_ID_{50} per 2 ml increased the overall mean level of exposure by five-fold, while doubling the volume of milk consumed daily and assuming all herds are infected had a lesser effect.

It’s perhaps not surprising in the sensitivity analysis with all herds positive, that the mean level of exposure for the positive exposures was relatively unaffected compared to the baseline. This is because positive samples only come from positive herds so the mean level of exposure per serving is independent of the number of positive herds. However, the proportion of positive samples was increased from 42% to 63%. Thus assuming all herds are positive means that 1.5-fold more daily exposures per person are positive but the average level of exposure per positive exposure is unaffected. This is reflected in a 1.5-fold increase in the overall mean level of exposure across all raw milk servings.

Assuming all cows in a positive herd were infected increased the overall (i.e. positive and negative exposures) mean level of exposure by nine-fold relative to baseline, while assuming all cows in all 81 raw milk herds in England and Wales were positive increased the overall mean level of exposure (i.e. positive and negative exposures) by 18-fold.

Table 10: Summary of results of sensitivity analyses

	Base line	Outbreak scenario	Baseline + 1 month shedding	Baseline + Including 20,000 GP_IP_ID_{50} per 2 ml maximum in raw milk	Baseline + Doubling daily raw milk consumption	Baseline + All herds positive	Baseline + All cows infected in a positive herd	Baseline + All cows positive
[1]% positive exposures	42.03	54.53	10.5	41.22	42.29	62.71	54.24	95.95
[2]Mean positive exposures only	1,265.7	11,612	411.5	6,736.7	2404.6	1,241.2	8,918.3	10,022.21
[2]Mean all exposures (i.e. positive and negative)	532.0	6,331	43.13	2,777.5	1,016.9	778.3	4,837.5	9,616.7
2.5th percentile	2	342	1	3	4	2	240	110
97.5th percentile	7,524	54,605	2,290	22,359	13,517	7,940	45,743	50,412

[1]Exposures are per person per day

[2]Units of GP_IP_ID_{50} per person per day

6. **DISCUSSION**

The simulated exposures suggest that the larger proportion of daily servings of raw milk do not contain *C. burnetii*. Indeed in the simulation, here, almost 60% of daily servings would be negative. That the major proportion of the 127 g servings has zero *C. burnetii* reflects the fact that some herds were not infected and that in the case of smaller infected herds (H = 2,3 cows) all the component cattle, by chance, were not shedding on that day. However, those 40% of daily servings which were positive for *C. burnetii* give an average level of exposure of 1,266 GP_IP_ID_{50} (2.5^{th} and 97.5^{th} percentiles were 2 and 7,524 GP_IP_ID_{50} per person per day). Although these appear high, PCR results suggest levels of exposures to *C. burnetii* DNA may be higher still. Thus, the levels of *C. burnetii* in raw BTM milk on the basis of PCR data are at least three orders of magnitude higher than the numbers of GP_IP_ID_{50} predicted here (Section 4.3). 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[3] may comprise more than one bacterium such that multiple *C. burnetii* genomes are present in a GP_IP_ID_{50}.

With regard to the number of bacteria comprising a GP_IP_ID_{50}, 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) (Table 4) suggest there could be between 2 and 112 *C. burnetii* organisms per GP_IP_ID_{50} in milk. Thus the mean number of GP_IP_ID_{50} in milk is 197.5 per 2 ml (Table 4) which is 98.75 per ml. 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 (calculation not shown). Since the mean is typically between the median and the maximum it is suggested here that there are between 2 and 112 *C. burnetii* organisms per GP_IP_ID_{50} in milk. Guatteo *et al* (2007) used the PCR method to estimate titres in cows' milk by comparison of PCR results with those from solutions with a known *C. burnetii* concentration obtained by serial dilution of an external positive control.

Validation of the simulation with PCR data is not possible directly because of the above reasons. It is concluded, however, that the predictions of GP_IP_ID_{50} in BTM are not inconsistent with the PCR data for BTM. Thus if each GP_IP_ID_{50} comprised

[3]In comparison, 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.

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 7) and observed number of PCR copies/ml (Figure 6).

The use of GP_IP_ID_{50} (measured in raw milk, Table 4) in this simulation overcomes the problem of PCR data that some of the DNA may represent non-viable *C. burnetii*. Indeed in unpasteurised cheeses, up to 4-logs/ml of DNA were detected by PCR but the samples were not viable in mice (Eldin et al. 2013). However, 2 of 6 PCR-positive raw milk samples contained viable *C. burnetii* (in mice) as shown by Loftis et al. (2010). Thus while the major proportion of the DNA in milk may not be viable, some is viable.

The use of GP_IP_ID_{50} in this risk assessment raises the issue of how infectious they are to humans through the oral route. It is known that *C. burnetii* is much less infectious to humans through the oral route than the inhalation route, reflecting the higher number of target macrophages in the lungs. Back in the 1940s Fonseca et al. (1949) demonstrated high infection rates by *C. burnetii* in humans through intradermal challenge. 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 an 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 by intradermal in Fonseca et al 1949). Furthermore, the genotype of *C. burnetii* may be important in relation to human infection. Thus, 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 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).

Epidemiological data suggest risks from *C. burnetii* are low through raw milk. There have been no reported outbreaks of illness associated with raw drinking milk or cream in the UK for over 10 years. Even with the caveats on under reporting of foodborne disease and the potential for undetected sporadic cases, the absence of reported outbreaks gives some assurance that the current controls are mitigating the risks associated with consumption of raw drinking milk (FSA 2012, para 13.2).

The exposures (i.e. probability of exposure and level of exposure in positive exposures) estimated here in 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 (as given by the Bernoulli variable Positive_Milk) does so every day as discussed in Section 3.1.6.

2. Use of ELISA seroprevalences. The within herd prevalence (p_{Within_Herd}) and between herd prevalence (p_{Herd}) used from McCaughey et al. (2010) are based on seroprevalence by ELISA and therefore do not represent a snapshot, but overestimate the proportion of animals infected at any given time. This in part relates to the duration of shedding in milk by an infected animal and its rate of recovery.
3. Use of PCR data for $p_{Shedding}$ (Guatteo et al. 2012) assumes that DNA in milk from an infected cow does indeed represent viable *C. burnetii*.

In addition no allowance is made for decay in the milk although due to the environmental stability of the small cell variant of *C. burnetii* (McCaul and Williams, 1981), decay in milk over a period of days may be limited. In the sensitivity analysis, a "What if? scenario" is used to test the effect of shedding for just one month per year. The effect was to decrease the percentage of positive exposures (per person per day) by 4-fold (from 42.03% to 10.5%) and decrease the mean level of exposure for positive exposures (per person per day) by almost three-fold. The mean overall level of exposure (including both positive and negatives) was decreased by 12-fold.

In the outbreak scenario, the proportion of positive daily servings of raw milk increased from 42.03% to 54.5%. This increase probably 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 level of exposure per positive serving increased by nine-fold from 1,266 to 11,612.1 $GP\_IP\_ID_{50}$. The frequency distributions for the levels of exposure in the positive exposure samples in the baseline and outbreak scenarios are compared in Figure 10. The modal level of exposure per serving increased from about $10^{2.5}$ to 10^{4} $GP\_IP\_ID_{50}$. This reflects the increase in number of infected cows within a positive herd and the increase in the proportion of infected cows which shed *C. burnetii* in milk. Thus a lot more *C. burnetii* within a herd are going into the milk giving a roughly nine-fold increase in the mean level of exposure through a positive serving (Table 10). The mean level of exposure from both positive and negative exposures combined increased roughly 12-fold in the outbreak scenario compared to baseline.

It should be noted that the herd size (H) varies from 2 to 300 in the model and therefore the output values of $Mean\_Raw\_Milk_{C.burnetii/litre}$ from simulations of smaller herds may have more extreme values than those from larger herds. Thus in a herd of 2 cows, if one is a high shedder then its input is only diluted into 2 x V_i (~50 litres of milk). In contrast for a herd of 300 cows the input from a high shedder is diluted into 300 x V_i (~15,000 litres). That consumers are exposed to variation is realistic because the BTM from different herds is not mixed for cows' milk in England and Wales.

7. CONCLUSIONS

Here the numbers of *C. burnetii* in raw cows' milk have been simulated taking into account the prevalence of infection in UK cattle herds and accommodating differences in prevalence between herds of different size. According to the results of the baseline model, 42% of 127 g raw milk volumes (the cumulative daily consumption for whole milk per person by UK men and women in the 19 to 64 age group) contain *C. burnetii*. The 58% of daily exposures that are negative reflects the fact that not all herds are positive in the UK. It is concluded that in terms of intraperitoneal guinea pig infective dose 50% ($GP\_IP\_ID_{50}$) units, those 42% of positive daily exposures contain relatively high levels of infectivity. It should be noted that a $GP\_IP\_ID_{50}$ unit may represent a relatively low risk through the oral route, although there are no data to relate $GP\_IP\_ID_{50}$ units to risk of oral infection in humans.

Although *C. burnetii* abortion storms have not been reported in UK cattle, an "outbreak scenario" was simulated assuming higher within herd prevalences of infection together with a higher proportion of infected cattle shedding in milk. The proportion of positive daily exposures increased only slightly reflecting the fact that the between herd prevalence was not changed. However, the mean level of exposures in positive daily exposures increased by nine-fold, and the overall mean level of exposure (including both negative and positive exposures) increased by 12-fold.

A major source of uncertainty is the duration of shedding in milk by infected cattle. The baseline model assumed continuous shedding for every day of the year. In a low shedding scenario in which infected cattle only shed for 1 month of the year, not only were the percentage of positive daily exposures decreased by four-fold (from 42.03% to 10.5%) but also the mean level of exposure for positive daily exposures was decreased by three-fold relative to the baseline model. The reduction in the overall mean level of exposure (including both negative and positive exposures) was 12-fold. Assuming all cows in the 81 raw milk herds in England and Wales were infected increased the overall mean level of exposure by 18-fold relative to baseline.

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